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A DISCUSSION ON METHODS FOR BLOOD AMYLASE DETERMINATION*

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INTRODUCTION

There is a need for a rapid, accurate, reproducible and inexpensive method for the determination of serum amylase. During the past decade, several methods have been tried,[1-5] but none have proved as satisfactory, for various reasons which will be discussed in the course of this paper, as has the method of Comfort and Osterberg[5] with some modifications.

General Considerations

Amylase is a starch-splitting enzyme. It brings about a conversion of starch to dextrins and ultimately to maltose, a reducing sugar. When iodine is added to soluble starch, a blue color is the result. The determination of amylase activity usually depends either upon the time necessary for the blue color given with iodine to disappear, or upon the quantity of maltose formed in a definite period of time. The first method is called the Iodometric method, and the second method is referred to as the Saccharogenic method. Due to the fact that amylase is an enzyme, the following factors are of great importance and must be observed carefully in all procedures:

1. The temperature
2. The hydrogen ion concentration
3. The sodium chloride
4. The concentration and nature of substrate
5. The amount of serum
6. The time of incubation

*Registry Award: Read before ASMT, Louisville, Ky., June 1953.

Saccharogenic Methods

There are several disadvantages to the "Kibrick and Roger"[1] Method. The length of time necessary to run the determination is long. The procedure involves a long incubation period for the substrate and serum, the making of a protein-free filtrate after the incubation period, the running of sugar determinations and the calculations. Repeating the procedure, if necessary, means the consumption of an equal amount of time as has already been spent. Also, a large amount of glassware and equipment is necessary. It requires the daily preparation of the starch substrate. The handling of the anthrone reagent is too dangerous in crowded and short-handed laboratories. The latter is especially true if there are inexperienced technicians in the laboratory.

Another Saccharogenic method tried was that found in a manual used at Graduate Hospital of the University of Pennsylvania.[2] Unlike the above procedure, the starch substrate and the other reagents needed in this procedure are not difficult to prepare. The reagents keep fairly well. Nevertheless, the procedure is long. The filtrations are slow. Repetition, if necessary, would take long.

Occasionally, a lipemic serum is obtained in which the iodine-starch color appears to get bluer as time goes on during the incubation instead of lighter. Perhaps, in these cases, a starch-fatty acid complex is formed which does not break down easily and consequently, prevents the amylase from acting on the starch.[13] It would be advantageous in these cases to use a Saccharogenic[2] method rather than an Iodometric method for determining blood serum amylase.

For those who wish to use this procedure, the following modification is of great help.

Modification: After incubating 30 minutes at 40° C., add one milliliter of ⅔ N. sulfuric acid and mix. Then add one milliliter of 10% sodium tungstate and mix. Filter. The results obtained with this modification are similar to those obtained when using copper sulfate and sodium tungstate. It is more advantageous to use reagents which are already at hand. The filtration is more rapid and the filtrate is clearer.

The method of David Polowe[3] is fairly simple. It takes about 40 minutes to run. In order to interpret the results properly, one needs to run a normal person each time. One must use tubes of similar size and always centrifuge at the same number of revolutions per minute. It is merely a qualitative test.

The following chart shows the similarity of results obtained by the two different filtrates.

CHART #1

Case No.	5% Copper Sulfate Plus 6% Sodium Tungstate	2/3 N H_2SO_4 Plus 10% Sodium Tungstate
1.	81 mgs. % glucose	83 mgs. % glucose
2.	33 mgs. % glucose	36 mgs. % glucose
3.	52 mgs. % glucose	60 mgs. % glucose
4.	360 mgs. % glucose	330 mgs. % glucose
5.	118 mgs. % glucose	115 mgs. % glucose
6.	414 mgs. % glucose	400 mgs. % glucose
7.	93 mgs. % glucose	101 mgs. % glucose
8.	211 mgs. % glucose	210 mgs. % glucose
9.	155 mgs. % glucose	160 mgs. % glucose
10.	148 mgs. % glucose	146 mgs. % glucose
11.	32 mgs. % glucose	37 mgs. % glucose
12.	58 mgs. % glucose	57 mgs. % glucose
13.	59 mgs. % glucose	59 mgs. % glucose

Iodometric Methods

The method of Somogyi[4] was a quantitative procedure used in our laboratory with great satisfaction and success for many years. Many results were followed up with surgical reports and were found to be accurate. A 100-watt frosted bulb in a carton box containing a small slit was used for viewing the end point. The starch solution, however, is rather tedious to prepare, and the calculations difficult for an inexperienced technician. The end point is a little difficult to determine, especially for someone not too familiar with it.

The Method of Comfort and Osterberg[5] has been found by far the most advantageous up to this date. The following modifications were made in our laboratory which simplified the procedure even further.

Modification: Four cubic centimeters of starch substrate are measured into a 16 x 100 mm. test tube in a water bath at 40° C. One cubic centimeter of serum is added to the substrate and the stop watch started immediately. Before adding the serum to the substrate, 0.5 ml. of 0.002N iodine solution is placed into each of several depressions of a white porcelain spot plate. (Coors porcelain, white plate with twelve depressions.) Immediately after adding serum to the substrate and mixing, a drop or two of the starch-serum mixture is added to the first depression containing the iodine solution. This first test gives the technician an indication as to the amount of amylolytic activity, and also an indication of how good the reagents are. Having an idea about the amount of amylolytic activity present, helps the technician determine how frequently to withdraw a portion of the starch-serum mixture for testing. Proceeding as indicated above helps the technician save on serum-starch mixture and helps one from running out of mixture. Upon approaching the end point, the full amount of 0.5 ml. of starch-serum mixture is added to the 0.5 ml. of 0.002N iodine. Proceeding as above eliminates the use of many tubes,

the shortage of serum-starch mixture, especially in normal serums, and the difficulty of detecting the end point. A small beaker is used for a water bath. (Slides showing procedure and equipment available.)

The following are two of the many cases of acute pancreatitis studied in our laboratory by this method.[5]

CHART #2

CASE #1

Amylolytic activity on admission with onset of pain—3200 units.

32 hours after the onset of pain, amylolytic activity was 1600 units.
40 hours after the onset of pain, amylolytic activity was 533 units.
62 hours after the onset of pain, amylolytic activity was 145 units.
82 hours after the onset of pain, amylolytic activity was 84 units.
9 days after the onset of pain, amylolytic activity was 94 units.

CASE #2

Few hours after onset of pain and vomiting, amylase activity was 1600 units.

12 hours after the onset, amylase activity was 640 units.
4 days after the onset, amylase activity was 228 units.
8 days after the onset, amylase activity was 200 units.

Chart #3 shows a study to determine the relationship between the Saccharogenic Method[2] and the Iodometric Method.[5] In both cases, our own modifications were used.

CHART #3

Case No.	Saccharogenic[2] Method mgs. of Glucose per 100 ml. of Serum	Iodometric[5] Method Units of Amylase Activity	Interpretation	
			Saccharogenic Method	Iodometric Method
1	97 mgs. glucose	200 units	N	N
2	59 mgs. glucose	230 units	N	N
3	83 mgs. glucose	160 units	N	N
4	70 mgs. glucose	178 units	N	N
5	36 mgs. glucose	below 80 units	N	N
6	60 mgs. glucose	178 units	N	N
7	321 mgs. glucose	1,070 units	A	A
8	115 mgs. glucose	145 units	N	N
9	87 mgs. glucose	76 units	N	N
10	400 mgs. glucose	530 units	A	A
11	80 mgs. glucose	100 units	N	N
12	101 mgs. glucose	178 units	N	N
13	43 mgs. glucose	80 units	N	N
14	201 mgs. glucose	320 units	B	B
15	165 mgs. glucose	230 units	N	N
16	146 mgs. glucose	200 units	N	N
17	37 mgs. glucose	below 80 units	N	N
18	57 mgs. glucose	below 80 units	N	N
19	57 mgs. glucose	160 units	N	N
20	360 mgs. glucose	640 units	A	A
21	193 mgs. glucose	160 units	N	N
22	252 mgs. glucose	640 units	A	A
23	256 mgs. glucose	640 units	A	A

Key:— N—Normal. A—Abnormal (Elevated). B—Borderline.
Note: Normal level for Saccharogenic[2] Method is 70—200 mgs. % glucose.
Normal level for Iodometric[5] Method is 0—320 units.

DISCUSSION

There is little doubt that the method of Comfort and Osterberg[5] with the modifications made in this laboratory has proved by far the most satisfactory procedure for the determination of serum amylase to this date. Most important of all has been the fact that the accuracy of the test with reference to the pathology has been excellent. The results can be available within a relatively short time after the blood has been drawn. In most acute cases of pancreatitis, the results have been obtained in a few minutes once the blood has clotted. The amount of blood required is small, and there is little trouble with quantities for repetition when necessary. The range is wide enough to cover abnormalities except for extremely high ones without subjecting the serum to any other treatment, e.g., additional dilutions. The preparation of the starch substrate is simple, and if kept free from mold and in the ice box at 4° C., will keep for several weeks.

There are no reagents of such a nature as to be dangerous for the technician to handle at any time. The results are easily reproduced. The procedure can be carried out easily, quickly, and accurately by someone who is not too familiar with the procedure.

The amount and cost of equipment and reagents, and the space required are negligible.

Because of the above reasons, the procedure is one of little cost to the hospital and especially to the patient, and a valuable one for application in a clinical laboratory.

In our laboratory, we have found that beef serum has a high amylolytic activity and makes excellent material for students to practice with. The beef blood can be obtained from a slaughter house and the serum collected after centrifuging.

SERUM AMYLASE

Reference: M. W. Comfort and A. E. Osterberg, **The Medical Clinics of North America,** Volume 24, Number 4, pp. 1137-1149, July, 1940.

Principle: Amylase in serum hydrolyzes a starch solution. After incubation of the starch-serum mixture at 40° C. for a definite period, it is added to a 0.002 Normal Iodine Solution to determine the end-point, which is indicated by a pure brown color or **absence** of blue.

Reagents:

STARCH SOLUTION: Triturate in a mortar, EXACTLY 75 mg. of C. P. Baker soluble powdered starch (Baker's Analyzed for Iodometry) and 250 mg. of C. P. Sodium Chloride. Add 10 ml. of cold water and pour it into a beaker containing 60 ml. of boiling distilled water. (Rinse the mortar with several small portions of distilled water, adding washings to the beaker). Transfer **quantitatively** to a 100 ml. volumetric flask. Cool. Bring to mark with distilled water. Keep in ice box at 4° C. If kept free from mold will keep quite some time (one month or more). Longer boiling will weaken starch solution. Do not make too large quantities. Prefer to make it about once a month.

0.1 NORMAL IODINE STOCK: Weigh accurately 1.2750 gm. of reagent grade iodine and transfer it quickly into a solution of 3.6 gm. of

C. P. Potassium Iodide in 10 ml. distilled water. After solution is complete, transfer quantitatively to a 100 ml. volumetric flask and dilute to the mark with distilled water. IF CAREFULLY PREPARED, NOT NECESSARY TO TITRATE. KEEP IN BROWN BOTTLE IN REFRIGERATOR.

DILUTE IODINE, 0.002 NORMAL SOLUTION IN 2% SOLUTION OF POTASSIUM IODIDE: Transfer 10 gm. of reagent grade potassium iodide to a 500 ml. volumetric flask. Add sufficient distilled water to dissolve the Potassium Iodide. With a 10 ml. volumetric pipette, add 10 ml. of N/10 iodine solution. Bring up to the mark with distilled water and mix thoroughly. IF CAREFULLY PREPARED, NOT NECESSARY TO TITRATE. KEEP IN DARK BOTTLE IN REFRIGERATOR. Keeps well.

Normal Levels: For serum up to 320 units.
Procedure: Technique **modified** from original.

1. Place a 16 x 100 mm. test tube containing 4 ml. of the starch solution in a water bath at 40° C. (can use a beaker with thermometer and add hot water from a tap, but keep temperature constant, ±0.5° C.).
2. Put 0.5 ml. of 0.002 Normal Iodine Solution in each of 12 depressions of a white Coors porcelain plate.
3. Add 1 ml. of serum to the starch in the bath. Use a 1 ml. pipette, subdivided in .01 ml. divisions and graduated to tip. Mix quickly by blowing up and down, and leave pipette in the tube. Start timing immediately.
4. In less than ½ minute, add a drop of starch-serum mixture to first depression containing iodine. Repeat at regular intervals until end-point is reached. Can use only a couple of drops of starch-serum mixture, until near end-point, **then** add full ½ ml. of starch-serum mixture to ½ ml. of 0.002 Normal Iodine Solution.
   a. The end-point is the point **just before** the complete lack of blue-black color. The next minute shows no blue-back color, but various shades of pale yellowish-brown color.
   Note the time in minutes it takes to reach the end-point. If amylase is very high, repeat using 0.5 ml. serum and replace starch with physiological saline. In this case, use time in minutes divided by 2 in the calculations.

Calculation:

$$\frac{1600 \text{ (starch factor)}}{\text{MINUTES TO REACH END-POINT (T)} \times \text{VOLUME (V)}} = \text{Units of amylase}$$

Suggestion: Use beef or dog serum to practice with or for students. Both serums have high amylases.

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NEWER DATA OBTAINABLE FROM THE BLOOD FILM: METHODS OF EXAMINATION AND SIGNIFICANCE OF FINDINGS*

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The blood film represents a "biopsy" of a living tissue and much may be learned from a careful study. On the unstained film are, besides the red blood cells, white blood cells and platelets, also the dried remnants of the proteins, fats, carbohydrates, salts, hormones, and other substances of the circulating blood.

The first essential is a *good* blood film. The "cover glass technic" is used, substituting slides for cover glasses. The slides are cleaned by dipping into isopropyl alcohol and then rubbing vigorously with a clean towel. A drop of blood is picked up (from an acetone-cleaned puncture in the finger tip) on the under surface of the *end* of a slide. This is touched to the *side* of another slide, the drop is pulled across (the slides being at right angles to each other) and then the flat surfaces are allowed to come in full contact, allowing the blood to spread between the two surfaces. The slides are then slowly separated. Two films may be made on each slide. (See Fig. 1.)

When dry, the slides are examined under low power magnification, with the light cut down to the maximum extent. Between the red blood cells minute points appear—hemokonia or chylomicrons. These may be lipoids, neutral fats or cholesterol. They are intensely increased in leukemia, polycythemia, myxedema, and hypercholesterinemia. Open spaces between the red blood cells, especially on the edges of the film, may contain

* Read before ASMT, Louisville, Ky., June, 1953.

these granules (normal) or they may contain one very large lipoid globule, much larger than a red blood cell. If care was taken not to pick up fat globules from the skin (extremely difficult when the ear is used) then these globules suggest defective emulsification of fat, and indicate an inadequate secretion of bile. (The condition may be corrected by giving the patient medication to increase the secretion of bile.)

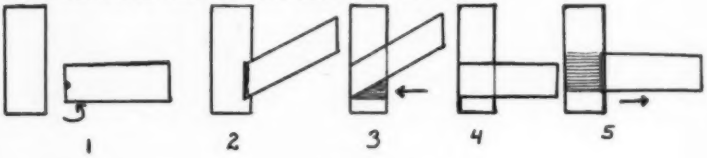

Figure 1

Method of Making Blood Films.

1. **Drop of blood on under surface of end of drawing slide.**
2. **Drop is allowed to spread.**
3. **Drawing slide is pushed across, held at about 45°.**
4. **Two slides allowed to come in contact until blood spreads.**
5. **Upper slide is drawn back, keeping both slides parallel, not at an angle to each other.**

When the globules are large and clear cut, the body increases the production of monocytes to pick them up. Under these circumstances, one finds a monocyte percentage above 7 per cent and vacuolated monocytes are present. Thus an increase in the number or percentage of monocytes with vacuolated forms, suggests a further study of the gall bladder and bile secreting mechanism. If the monocyte number is increased, and there are no vaculated forms, the disease is usually in the pelvis, or the patient may have malaria.

Another feature secondary to defective emulsification of fat and bile deficiency is the hydrolysis of unabsorbed fat in the intestine with the subsequent absorption of "fatty acids." In this case, the red blood cells become spherical or semi-spherical instead of biconcave, and hemolyse easily. This may be demonstrated by placing a pen mark, an "X" or an "O" on the *stained* film, using an ink like Skrip. If the cells are biconcave, a droplet of ink will appear like a large nucleus in the red blood cells. If they are spherical, no ink will appear in the center. In semi-spherical forms, a very small droplet of ink appears in the middle of the corpuscle. (See Fig. II, 1 and 2)

When abnormal proteins are present, in increased amount or in abnormal proportions, the dried residue may be noted in the unstained film, occasionally after it is stained.

If a beam of light is sent through the unstained film, it is broken up into a spectrum, with concentric rings. All the normal colors are represented, blue being innermost, then green, yellow,

orange, red. If there is an increase in blood bilirubin, the blue overlaps the green, in proportion to the increase in the bilirubin.

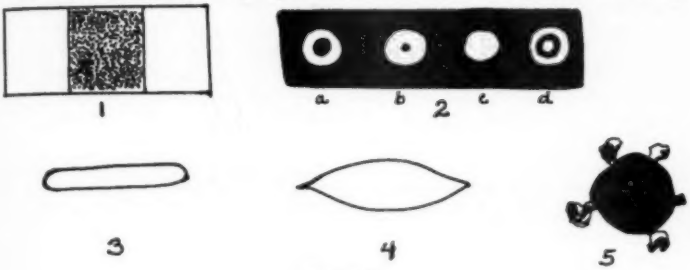

Figure II

1. **A mark (X) is made with ink on the stained film.**
2a. **Normal red blood cell with ink in the hollow in the center.**
2b. **Semi-spherical red blood cell. Very little ink in the hollow.**
2c. **Spherical red blood cell. No central mass of ink.**
2d. **Target red blood cell. Ink is in the groove around the central area.**
3. **"Pencil" red blood cell. (Chronic hemorrhage.)**
4. **"Amphioxic" red blood cell. (Acute hemorrhage.)**
5. **"Exploded" red blood cell in peripheral blood. (Crowded bone marrow.)**

For staining, Wright's stain, followed by distilled water, gives adequate results. The Wright's stain may be tested by drying a drop on a piece of filter paper and then adding several drops of distilled water. If the stain is good, a red ring will develop around the blue center. In the stained film an estimate of the "color index" may be made from overlapping red blood cells. A normal red blood cell with a color index of 1 will be so opaque that the outlines of an underlying cell will not be discernable. If the outline of the underlying cell can be made out, the color index is less than 1 and there is an iron deficiency. However, if the cells are spherical they may be opaque because of their increased thickness.

Rouleaux formation is important, because it is the basis of the sedimentation rate. If there is much rouleaux formation, one may estimate that the sedimentation rate is rapid. In anemia the red cells settle more rapidly in a test tube, not because of a disease process, but because the cells having less resistance to falling because of their decreased number, settle more rapidly than normal. The clue to the significance is given by the fact that rouleaux formation is not increased.

Autoagglutination of the red blood cells in the film is of diagnostic importance. Here the cells group into compact masses of 2, 3, 4, or more. This is characteristic of multiple myeloma, neoplasms, Hodgkin's disease, and occasionally in severe sepsis.

The shapes of the red blood cells are of considerable diagnostic

importance. The oblong macrocytes of pernicious anemia are familiar, the spherical and semi-spherical forms of hemolytic anemia, poikilocytes in conditions in which there is gross disturbance in blood formation, "pencil" shaped red blood cells in chronic hemorrhage, "exploded" forms when the marrow is crowded, amphioxic (bi-pointed) forms in acute hemorrhage, target cells in liver disease, and certain blood disturbances, sickle forms in sickle cell disease, and oval forms in ovalocytosis. (See Fig. II, 3, 4 and 5.)

The diameter of the red blood cells varies with certain conditions. The normocytic anemias include aplastic anemia and certain nephropathies. Large oval cells with microcytes are found in pernicious anemia, whereas large round cells, without microcytes characterize the blood of sprue, myxedema, carcinoma of the head of the pancreas, intestinal anastomosis, certain liver diseases, certain cases of carcinoma of the stomach, and when the reticulocyte count is high. The large red blood cells in these conditions are well colored. Pale, large red blood cells suggest swelling, as in the last three months of pregnancy and in conditions in which the blood is "diluted." Small red blood cells, when pale, suggest the result of hemorrhage, but when well colored, they suggest the spherical cells of hemolytic anemia. Cells with extremely small diameters are found in polycythemia vera.

Structures in the red blood cells are important. There are malarial parasites and rickettsia. In conditions in which the spleen is inactive or has been removed, there are nuclear particles and Howell-Jolly bodies. Reticulum substance, granules and nuclei mark immature forms; Heintz bodies in certain poisonings; basophilic stippling in severe anemias and certain heavy metal poisonings and in some disturbance of hematopoiesis, acidophilic stippling and Cabot rings.

From the white blood cells much information can be gathered as to the adequacy of the bone marrow (mature or immature forms), or the presence of pyogenic infection or absorption of products of tissue decomposition (basophilia of the granules of the neutrophiles), or virus infection (blue staining, or red staining granules in the lymphocytes from the spleen). Eosinophiles suggest a group of possibilities, and even 1 per cent may be considered significant. The special cells of infectious mononucleosis, the phagocytic endothelial cells of subacute bacterial endocarditis and similar lesions near large blood vessels (phlebitis), are of course diagnostic. Plasma cells indicate involvement of lymph nodes in the disease process, or multiple myeloma. Kupffer cells, mistaken for lymphocytes, indicate liver involvement, as does also vacuolated monocytes (bile deficiency). Neoplasm cells, occasionally mistaken for lymphocytes are of great diagnostic importance. Auer bodies in blasts indicate myelogenous or monocytic leukemia.

The number of platelets can be estimated only on a film made from blood mixed with brilliant cresyl blue. It is of importance to note if the platelets are unusually large (hemorrhagic tendency).

There are much more data that can be learned from the blood film, and the field is open for future developments.

TECHNIQUES FOR THE ISOLATION AND IDENTIFICATION OF ENTERIC BACILLI*

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The enteric bacilli may be defined as gram-negative, non-spore forming bacilli which grow well on artificial media. These organisms are identified by their cultural characteristics, biochemical reactions and frequently by their antigenic structures. Some of these bacilli are considered to be a part of the normal flora of the intestinal tract, whereas others are present only in an infectious process. The isolation and identification of these organisms is important, not only for the immediate diagnosis, but an accumulation of such data concerning the isolation of these organisms from the normal and diseased intestine will determine the significance of these bacteria.

The technique which is presented has been employed in an extensive study of infantile diarrhea. This procedure has proved satisfactory because it is rapid and accurate, yet it is practical in the small laboratory.

Materials and Methods

The procedure followed in the isolation and identification of the enteric bacilli is presented in Figure I.

I

Specimen: The specimen desired is a rectal swab.[5] The swab is easily prepared by inserting a cotton tipped applicator through a piece of soft rubber tubing (3/16" x 3") until the tip reaches the beveled end of the tubing. The swab is placed in a plugged test tube and sterilized by autoclaving for 20 minutes at 121°C (15 lbs. pressure).

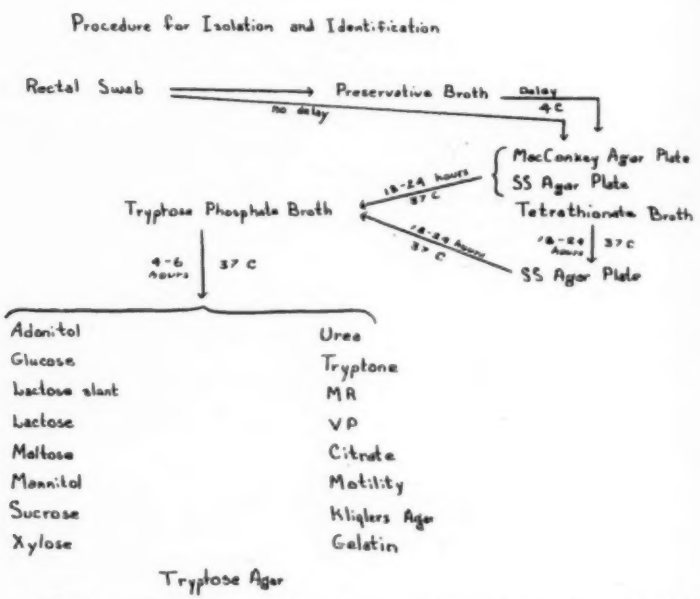
Preservative broth: If there is to be a delay between the time the specimen is taken and the media are inoculated, the

* Read before Texas Society of Medical Technologists.

swab is placed in a liquid medium which prevents the overgrowth of the coliforms and keeps the specimen moist. Refrigeration of the specimen is desirable.

Plating media: When the specimen is received in the laboratory it is removed from the preservative medium and streaked over one-third of the surface of a MacConkey agar (Difco) and on one-half of a SS agar (Difco) plate. Utilizing the entire surface area, the plates are streaked for isolated colonies. These plates are incubated at 37°C until the following morning.

Figure 1



Enrichment medium: After the plates have been streaked with the rectal swab, the applicator is placed in tetrathionate broth. This culture is incubated at 37°C overnight, after which time a subculture is made on a SS agar plate.

Isolation: After 18-24 hours incubation the plates are examined for the presence of colonies of non-lactose fermenting organisms. These are easily detected by the colorless, white, or slightly pink colonies. On SS medium many of the hydrogen sulfide producing bacilli will produce colonies having grey or black centers. At least one colony of each colonial type present is isolated.

Using a flamed loop or needle, an isolated colony is inoculated to tryptose phosphate broth containing brom-thymol blue indicator. This culture is incubated at 37°C until growth is visible. Growth, which is usually good within 4-6 hours, is detected by turbidity and the production of acid as indicated by the color change.

Identification: A series of 17 liquid and solid media are used in the identification of each organism isolated. Using a 1 ml. pipette, the broths, ten per cent lactose slant and tryptose agar slant are inoculated with a drop of the tryptose phosphate broth culture. The surfaces of the citrate and gelatin slants are each inoculated with a single stroke of the inoculating needle and the sulfide and motility test media are inoculated by a single, central stab. The series is then incubated overnight at 37°C.

The tests are read first after 18-24 hours incubation. To prevent later confusion, the tubes showing a positive reaction are discarded when the readings are made. If necessary, the indol test may be performed at this time, although the procedure is simplified if all of the tests requiring the addition of test reagents are read and discarded after 48 hours. It is desirable to read the remaining tests daily until they are discarded at the end of seven days.

The serological identifications of the shigellae and salmonellae are made at the time of the first reading. When an isolate exhibits the cultural and biochemical characteristics of *Shigella* or *Salmonella*, the growth from the tryptose agar slant is used to prepare an antigen. The growth may be suspended first in saline or suspended directly in the drop of typing serum on the glass slide. A "control" serum is included to rule out the possibility of a rough antigen. The antigen-serum mixture is mixed well and the slide is gently rocked. Agglutination, or clumping, indicates a positive reaction.

Preparation of Media

Preservative broth: The preservative broth described by Felsenfeld[3] is employed. This broth has the following composition per liter:

sodium citrate	10 gm
sodium peptone No. 3	10 gm
Bacto-oxgall	6 gm
sodium chloride	9 gm
dibasic sodium phosphate	3 gm
para-aminobenzoic acid	.05 gm

The medium is tubed in 3-4 ml. quantities and refrigerated until used.

Plating Media: The MacConkey and SS agars are prepared for use according to the manufacturer's instructions and poured

into sterile petri dishes when sufficiently cooled to prevent the formation of excessive water of condensation. The best differentiation is obtained when each plate contains 20-25 ml. of medium.

Tryptose phosphate broth: The tryptose phosphate broth (Difco) is rehydrated according to the directions. One ml. of a 1.6 per cent solution of brom-thymol blue in alcohol is added to each liter of medium. The broth is tubed in 4 ml. amounts in 13 x 100 mm. sterile test tubes. The broth is autoclaved for 10 minutes at 118-121°C and cooled immediately.

Carbohydrate broths: The carbohydrates included in the fermentation studies are adonitol, glucose, lactose, maltose, mannitol, sucrose and xylose. Each broth is prepared by the addition of the carbohydrate in a concentration of 0.5 per cent to purple broth base (Difco). The broths are tubed in 3.0-3.5 ml. quantities in 13 x 100 mm. sterile test tubes. Fermentation vials are used only in the glucose and lactose broths. The tubes of media are placed in wire racks and autoclaved for 10 minutes at 118-121°C. It is desirable to pre-heat the autoclave to reduce the time required to reach this temperature. After 10 minutes of autoclaving, the pressure is released as rapidly as it is possible to do so without causing the plugs to be blown from the tubes. When the pressure has been released, the racks are removed from the autoclave immediately and placed in trays of iced water.

Urea broth: The concentrated urea broth (Difco) is added to sterile distilled water and tubed in 1.0-1.5 ml. quantities. The use of urea concentrate eliminates the problems of filtration.

Solid media: The ten per cent lactose slant is prepared by the addition of lactose to the purple agar base (Difco) and tubed in 1.5-2.0 ml. quantities. After the medium has been autoclaved for ten minutes at 118-121°C the tubes are slanted in such a manner as to prevent the formation of a butt.

Simmon's citrate agar and Stone's gelatin agar are rehydrated and tubed in 1.5-2.0 ml. amounts in sterile 13 x 100 mm. test tubes. The sterilized media are stored in the refrigerator as "deeps" and melted just prior to use to insure having a fresh slant.

The motility test medium (Difco) and Kligler's agar (Difco) are rehydrated and tubed in 13 x 100 mm. sterile test tubes. The tubes are filled one-half full and after autoclaving are allowed to solidify in an upright position. If desired, the Kligler's agar tubes may be prepared with a slant providing a deep butt is retained.

Tryptose agar (Difco) is prepared and tubed in 4-5 ml. amounts in a test tube (⅝ x 6") large enough to give sufficient surface area for the antigen production. These tubes are stored as "deeps" and slanted just before using.

The medium employed for maintaining the enteric bacilli in the stock collection is a beef-infusion broth to which 0.15 per cent agar is added. Brom-thymol blue indicator is added to detect any pH change which may influence the survival of the culture.

Biochemical Tests

Indol test: The test for indol is performed by the addition of a few drops of Kovac's reagent to a one per cent tryptone (Difco) broth culture of the organism being studied. The tube is then shaken slightly to allow the extraction of indol. The layer of reagent becomes red when indol is produced. This test may be performed at 18-48 hours.

Kovac's reagent

para-dimethyl-amino-benzaldehyde (Pfansteihl)	5 gm
normal butyl alcohol	75 ml
concentrated hydrochloric acid	25 ml

The reagent will deteriorate less rapidly if refrigerated.

Methyl red test: The methyl red test is made by the addition of a few drops of methyl red indicator to a 48 hours old culture of the organism in MR-VP medium (Difco). A positive reaction is indicated by a red color. The indicator solution is prepared by dissolving 0.01 gram methyl red in 20 ml. of 95 per cent ethyl alcohol and diluting to 50 ml. with distilled water.

Voges-Proskauer test: One ml. of the MR-VP broth culture is used in determining the production of acetylmethylcarbinol. The test is performed by the addition of 0.6 ml. of reagent 1 and 0.2 ml. of reagent 2. The test is shaken and read after 30 minutes. The production of a red color indicates a positive reaction.

Reagent No. 1

Alpha naphthol	5 gm
Absolute alcohol	100 ml

Reagent No. 2

Sodium hydroxide	40 gm
Creatine	0.3 gm
Distilled water	100 ml

Gelatin liquefaction test: The liquefaction of gelatin is determined by the addition of a saturated solution of ammonium sulfate to the culture on the gelatin medium. When gelatin is liquefied there is no change in the appearance of the medium. If the test is negative the medium becomes white due to the precipitation of the gelatin.

Discussion

This procedure for the isolation and identification of enteric bacilli is designed to increase the number of isolations and to give a rapid, accurate identification of the organisms isolated. Within

thirty-six hours after the specimen is received, it is possible to have a tentative and frequently a positive identification of the bacilli isolated on direct plating.

In recent years the use of the rectal swab[5] has become almost universal. The reasons for its popularity are obvious: (1) the specimen can be obtained when desired, (2) the specimen is more pleasant to handle, (3) the disposal problem is eliminated and (4) the results are as good as, and frequently better than, those obtained with a stool specimen.

The use of a preservative medium is desirable if there is to be a delay between the time a specimen is collected and it is plated. This broth not only keeps the specimen moist; it prevents the overgrowth of the pathogens by the more rapidly growing organisms. The medium described by Felsenfeld[3] is designed to maintain the flora unchanged. This is advantageous to those studying the bacteriology of the normal and infected intestine.

MacConkey and SS agars were selected for the plating media because they differ in their selective properties. SS agar inhibits a majority of the coliforms, thus allowing the use of a larger inoculum than can be used on the MacConkey agar, which inhibits only the gram-positive bacteria. It is not uncommon to observe only one or two non-lactose fermenting colonies on the MacConkey agar plate, whereas their growth will be heavy on SS agar. Nevertheless, the MacConkey agar plate must be included in the procedure because some of the organisms present may be isolated on this medium but inhibited by the SS agar.

The use of enrichment broths in addition to two plating agars increases the number of isolations, especially of the *Salmonella* types. The enrichment media, such as tetrathionate, inhibit many of the coliforms, thus allowing the development of the salmonellae, which may be present in small numbers in the carrier or convalescent. Few of the shigellae survive in enrichment broth and the isolation of these organisms is dependent on direct plating procedures.

The use of double or triple sugar agars for the identification of the *Enterobacteriacae* is almost universal. These media usually prolong the identification by at least 24 hours and frequently lead to confusion and a waste of expensive typing serum. On Kligler's agar, which is one of the most widely used, the *Shigella* species, *Proteus rettgeri*, anaerogenic paracolons and frequently *Salmonella typhosa* exhibit the same behavior. A difference in odor is usually observed in these organisms but this character is not a reliable one. The production of acid and gas in the butt with an alkaline slant is the typical reaction of *Proteus morganii*, some of the paracolons, and hydrogen sulfide negative *Salmonella* types. The hydrogen sulfide producing bacilli present an equally confusing picture. In order to differentiate the *Shigella* and *Salmonella* types from these other

bacilli isolated on the multiple sugar medium, it is necessary at this time to use antiserum. Even though a positive agglutination is obtained, it is necessary to identify the isolate further by its fermentation reactions as some of the paracolons possess *Shigella* or *Salmonella* antigens.

Since the identifications of the enteric bacilli are based on the fermentation patterns, the preparation of media containing carbohydrates is very important. Care must be taken to prevent the hydrolysis of the disaccharides by excessive heating. Although this danger may be avoided by the aseptic addition of sterile solutions of the carbohydrates to tubes of sterile broth, this procedure is time consuming and tedious. If certain precautions are observed these media may be autoclaved. The sterilization time may be reduced to a minimum of eight to ten minutes at 118-121°C if the media are added to previously sterilized test tubes. It is desirable to pre-heat the autoclave to obtain the temperature of sterilization in the shortest time possible. By placing the tubes in racks the steam is evenly distributed around the tubes and after autoclaving the tubes can be cooled quickly by the circulation of cold water around each tube. It is impossible to exaggerate the importance of quickly attaining the sterilization temperatures and the rapid cooling of the sterile media.

Purple broth base (Difco) is used for the preparation of the carbohydrate broths. The indicator in this product, brom cresol purple, is recommended for the determination of fermentation by the enteric bacilli because the change from purple to yellow occurs at a pH of 6.3. This hydrogen ion concentration is attained only after the fermentation of the added carbohydrate. The use of slightly acid water or the fermentation of a trace of glucose present as a hydrolysis product will not alter the pH of the medium sufficiently to produce a color change and give a false positive reading.

Table 1 shows the behavior patterns established for the enteric bacilli when inoculated in the series of media recommended for use in their identification. Each fermentation and biochemical reaction serves to establish the genus of the isolate and to determine its species.

Adonitol: This pentahydric alcohol is used especially in the identification of *Proteus rettgeri*. Although it may be omitted from the "routine" series, adonitol should be used for the identification of atypical strains of *Proteus rettgeri*.

Glucose: With the exception of *Pseudomonas* and *Alcaligenes*, all of the aerobic gram-negative bacilli which may be isolated from the intestinal tract ferment glucose. The production of gas by the isolate is an important differential characteristic.

Ten per cent lactose slant: To detect the paracolons[2] rapidly a high concentration of lactose (10%) is included in this series.

TABLE I

Biochemical Reactions of Enteric Bacilli

	Adonitol	Glucose	Glucose: gas	Lactose: 10%	Lactose	Maltose	Mannitol	Sucrose	Xylose	Urea	Indol	MR	VP	Citrate	Gelatin	Motility	Sulfide
Proteus mirabilis	—	+	+	—	—	—	—	—	+	+	—	±	+	+	+	+	+
Proteus morganii	—	+	+	—	—	—	—	—	—	+	+	+	—	—	—	+	—
Proteus rettgeri	+	+	— (+)	—	—	—	+	— (sl +)	—	+	+	+	—	+	—	+	—
Proteus vulgaris	—	+	+	—	—	+	—	+	+	+	+	+	—	—	+	+	+
Salmonella typhosa	—	+	—	—	—	+	+	—	+	—	—	+	—	+	—	±	sl +
Salmonella types	—	+	+	—	—	+	+	—	+	—	—	+	—	+	—	+	+
Shigella sonnei	—	+	—	sl + dgt	sl + (—)	sl +	+	— (sl +)	—	—	—	+	—	—	—	—	—
Shigella paradysenteriae	—	+	—	—	—	—	+	—	—	—	—	+	—	—	—	—	—
Escherichia coli	—	+	+	+	+	+	+	— (+)	+	—	+	+	—	—	—	+	—
Aerobacter aerogenes	+	+	+	+	+	+	+	+	+	—	—	—	+	+	—	—	—
Paracolobactrum coliforme	—	+	+	sl + dgt	sl +	+	+	— (sl +)	—	—	+	+	—	—	—	+	—
Paracolobactrum intermedium	—	+	+	sl + dgt	sl +	+	+	— (sl +)	+	—	—	+	—	+	—	+	+
Paracolobactrum aerogenoides	+	+	+	sl +	sl +	+	+	+	+	—	—	—	+	+	—	—	—
Alcaligenes	—	—	—	—	—	—	—	—	—	—	—	—	—	— (+)	— (+)	+ (—)	—
Pseudomonas	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—

sl—slowly. ( )—occasional reaction. dgt—daughter colonies.

The slow fermenters of lactose will frequently ferment lactose in this concentration more rapidly than in the 0.5% broth. The entire surface of the slant is inoculated for the detection of lactose fermenting daughter colonies.

Lactose: The fermentation of this carbohydrate has served as the basis of differentiating the coliforms from the other enteric bacilli since the early days of enteric bacteriology. At one time it was assumed that all lactose fermenting gram-negative bacilli were non-pathogenic.

Maltose: *Proteus vulgaris* can be quickly distinguished from *Proteus mirabilis* through the use of this disaccharide. Many of the *Shigella* species slowly ferment maltose.

Mannitol: The *Shigella* species most frequently encountered in the Southwest[3,4] are *Sh. sonnei* and various types of *Sh. paradysenteriae*, which ferment mannitol.

Sucrose: Some of the paracolon bacilli will ferment sucrose, differentiating them from the recognized pathogens. *Proteus vulgaris* is identified by its fermentation of sucrose.

Xylose: A more complete fermentation pattern is established by the reaction of the isolate in xylose broth. This pentose is especially valuable in the identification of atypical strains of enteric bacilli.

Urea: The utilization of urea as the source of nitrogen is an identifying feature of the *Proteus* species. The development of a pink color in the broth quickly distinguishes *Proteus* from *Shigella* and *Salmonella*.

Indol: Indol is produced when bacteria attack the side chain of tryptophane and do not utilize the indole ring. The peptone, tryptone (Difco), contains a high concentration of this amino acid and is recommended for the detection of indol production.[6]

This single test quickly rules out the possibility of the isolate being *Salmonella*. Although there are indol producing strains of *Salmonella*, these are extremely rare. If the fermentation pattern is suggestive of that of *Salmonella*, the indol test can be quickly performed. Certain paracolons are most likely to be mistaken for the hydrogen sulfide negative *Salmonella* and the majority of these paracolon bacilli are indol positive.

MR-VP: The methyl red test was first used as a means of differentiating the coli-aerogenes group of bacilli. In a buffered glucose broth the coli group produces a high degree of acidity, whereas the aerogenes group gives a more alkaline reaction on prolonged incubation.

Although an organism that gives a positive methyl red test usually fails to produce acetylmethylcarbinol, there are a few enteric bacilli which will give positive results in both tests.

The methyl red and Voges-Proskauer tests are included to

determine the species of *Paracolobactrum* and to establish the IMVIC* pattern of the other organisms.

Citrate: The utilization of citrate as the sole carbon source is a differential characteristic of the *Salmonella, Proteus rettgeri, Proteus mirabilis,* certain paracolons and coliforms. Simmons citrate agar depends on a color change to denote a positive reaction, thus making the test easier to read than the determination of turbidity in a liquid medium.

Gelatin: The primary value of gelatin is the identification of *Pseudomonas* and *Proteus.*

Motility: Flagella production, or motility, is more accurately determined by observing growth away from the line of stab in a clear, semi-solid medium. Since the production of flagella is sometimes better at 20-25° C., than at 37° C., it may be necessary to hold the culture at room temperature to obtain a positive reading. If the majority of the bacilli present are non-motile, the actively motile organisms may be detected by tufts of growth arising from the central stab line.

Sulfide: Kligler's agar is included only for the purpose of determining the production of hydrogen sulfide. A positive reaction is important in the identification of the majority of *Salmonella* types, *Proteus mirabilis, Proteus vulgaris* and some of the paracolons.

Antigen slant: A tryptose agar slant is inoculated with each series because it is desirable to have the antigen available if antigenic typing is indicated. Since the typing sera are expensive, it is practical for the small laboratory to use sera only to confirm the biochemical identification as to the genus of the organism. For a species or type identification the isolate should be submitted to a larger laboratory equipped for such studies.

Stock culture: When growth is detected in the stock medium the culture is sealed to prevent the drying out of the medium. Refrigeration is not necessary if the cultures are kept in a cool place.

Summary

A technique for the isolation and identification of the enteric bacilli is described. The procedure allows a more rapid and accurate identification of the isolates. Through the use of the methods discussed the identification of the organism is obtained at least 24 hours earlier than when the double or triple sugar agars are employed.

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* IMVIC: indol, methyl red, Voges-Proskauer, citrate.

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THE NEED FOR MORE THAN ROUTINE MICROBIOLOGY

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In the past 15 years we have witnessed remarkable progress in the application of microbiological procedures in clinical laboratories. More and more demands are constantly being placed on clinical laboratories for bacteriological studies with respect to diagnoses, certain phases of treatments, and progress of patients with infectious diseases. Unfortunately many laboratories have failed adequately to meet this demand and as a result have not only neglected to fulfill their responsibilities but have discouraged the continued interest of many physicians in obtaining competent bacteriological work.

In many instances the failure is due to the erroneous idea that medical microbiology can be fitted into a limited routine procedure. This limited routine procedure is frequently based on three popular misconceptions:

1. Certain bacterial species are always responsible for certain infections.

2. The use of two or three different media, theoretically endowed with the ability to grow any and all bacteria, is all that is required to culture the responsible agent from an infectious disease.

3. A gram-stained preparation of the specimen submitted to the laboratory is sufficient to identify the etiologic agent.

Adherence to such limited procedures has resulted in mediocre microbiology which is far below any acceptable standard. The need for adequate microbiological technique in a clinical laboratory cannot be over-emphasized. Despite the fact that many infectious diseases present a clinical picture which is often diagnostic, no acceptable diagnosis of an infectious disease can be made unless the causative agent is isolated and identified, or established by acceptable serological techniques. By following a flexible set of procedures designed to demonstrate as many bacterial and fungal species as possible in sus-

pected infections the laboratory can materially aid in the establishment of correct diagnoses. The relative merits of a complete microbiological routine over a limited routine can best be illustrated by citing a few cases from our own experience. In presenting these cases only the facts pertinent to this discussion are given.

Case 1. A 50 year old female entered the hospital with a large indurated area in the right inguino-femoral region. Outwardly it appeared to be a localized abscess. Surgical drainage revealed an extensive necrotizing fasciitis and what was called "gas gangrene." Anaerobic cultures for gas bacilli were requested. In addition to complying with the request for anaerobic cultures, aerobic cultures were also made from the swabs and portions of tissue from several sites of the affected area. Gram-stained preparations of smears from these regions showed many gram-positive cocci resembling both streptococci and micrococci. No gram-positive bacilli resembling the clostridia were seen. The initial culturing as well as several subsequent culturings failed to yield growth of any species of the genus *Clostridium*. In each case growth of a non-hemolytic streptococcus and *Micrococcus pyogenes* var. *aureus* was obtained. On the strength of the bacteriological findings, a diagnosis of progressive bacterial gangrene produced by a synergistic action of the streptococcus and the micrococcus was made. This disease was first reported by Cullen in 1924 and was later designated the Meleney syndrome after F. M. Meleney (Brewer and Meleney, 1926; Meleney, 1931, 1933) who demonstrated that strains of a non-hemolytic streptococcus and of *Staphylococcus aureus* isolated from cases similar to the one reported upon here would produce the same type of gangrene when injected simultaneously into experimental animals. Neither organism alone would produce the gangrene. The case described here bore striking similarities to those described by Meleney (1933, 1945, 1950) and by others (Grimshaw and Stent, 1945; Wooldridge and Hodgeboom, 1953) and, with the isolation and identification of the two bacterial species, the diagnosis of progressive bacterial gangrene seemed justified.

Case 2. A second case was that of an 87 year old man who complained of a painful, tender and swollen area on the right side of his neck just below the angle of the jaw. This lesion had the typical appearance of a pyogenic abscess. Stained preparations of the purulent exudate showed gram-positive cocci which after suitable culture were identified as *M. pyogenes* var. *aureus*. However, this infected area failed to heal after extensive antibiotic therapy and a more extensive examination of the lesion was conducted. Acid-fast stained preparations made from the deep necrotic material revealed acid-fast bacilli typical of *Mycobacterium tuberculosis*. This was confirmed by culture and

by histopathological studies. This case amply indicates that the outward appearance of a lesion and even the initial bacteriological findings may be misleading. This case also points out the value of frequent acid-fast stained preparations and cultures in chronic and recalcitrant infections.

Case 3. On three distinct instances in the past year patients complained of an abdominal mass and sought treatment for what was assumed to be a tumor. The data from one representative case will serve to illustrate that occasionally bacterial infections may simulate neoplastic growth. A 38 year old female was admitted to the hospital for removal of a pelvic tumor. At surgery, the previously palpable pelvic mass was found to be a large pelvic abscess. Aerobic cultures made from aspirated fluid submitted to the laboratory failed to yield any growth despite the fact that gram-stained preparations showed an abundance of gram-negative bacilli. Anaerobic cultures were made by inoculating deep tubes of thioglycollate broth and, in each instance, the resulting growth resembled that of *Bacteroides* sp. We feel that this is a particularly important finding because it indicates that perhaps all too frequently negative reports are issued from the laboratory when actually the report are based on incomplete laboratory work. The significance of *Bacteroides* infections is well attested to by several investigators (Beigleman and Rantz, 1949 and McVey and Sprunt, 1952) who maintain that *Bacteroides* infections should not be regarded as exotic and rare conditions.

Case 4. A 3 year old child with a bilateral infection of the auditory canals was reported by another laboratory as having a diphtheroid infection. The diagnosis was based entirely on examination of a gram-stained preparation of the sero-purulent exudate. The infection failed to respond to treatment with penicillin and the child was brought to the out-patient department to have the antibiotic sensitivity of the organism determined. Examination of the gram-stained preparations of the exudate showed gram-positive cocci which conceivably could have been called diphtheroids. However, cultures made from the exudate gave growth which proved to be pneumococci. Subsequent antibiotic sensitivity tests indicated that the strain of pneumococcus was relatively resistant to penicillin but was sensitive to the broad spectrum antibiotics. Here the danger of attempting diagnoses by examination of gram-stained preparations alone is clearly demonstrated.

Case 5. A 23 year old female was referred to the hospital for diagnosis and treatment by another hospital in the eastern part of the state. While at this other hospital, the patient complained of general malaise and weakness and had a diarrhea and a temperature of 104 F. The attending physician had, three weeks prior to admission, given the patient her annual booster shot

of typhoid vaccine. The agglutination titers of the patient's serum with *Salmonella typhosa* O and H antigens were 1:320. After admission to St. Joseph's Hospital an anemia and a slight leucopenia were also established. A repeat of the Widal agglutination gave an O titer of 1:1280 and an H titer of 1:160. Two blood cultures taken at three day intervals, two stool cultures and one urine culture all failed to yield growth of any enteric pathogen or other pathogenic organism. The serology was again checked and the initial titers were confirmed. Despite the previous immunization, the serology was indicative of active typhoid fever. Since the organisms could not be recovered from the peripheral blood, a sternal biopsy was performed and a portion of the bone marrow was cultured for enteric pathogens. The bone marrow cultures gave growth of a gram-negative bacillus which was identified biochemically and serologically as *S. typhosa*.

This one case taken alone clearly indicates the necessity for a flexible, open minded approach to clinical microbiology. This case also serves to illustrate the oft forgotten fact that induced immunization against typhoid fever is only relative and that the degree of immunity may not be able to neutralize an exposure to an overwhelming number of organisms. (Syverton, Ching, Cheever and Smith, 1946; Anderson and Richards, 1948; Morgan, 1952.)

Case 6. These same facts are emphasized in another case of typhoid fever. A 22 year old female was admitted for study for a possible adrenal gland tumor. Two weeks after admission, the patient was subjected to an exploratory operation. The gall bladder was found to be chronically inflamed and contained several stones. The gall bladder and stones were removed. Two weeks after the operation the patient's temperature spiked to 104 F. Blood cultures taken at this time showed growth of gram-negative bacilli which proved to be *S. typhosa*. Stool and urine cultures were negative for typhoid bacilli. The patient was successfully treated with chloramphenicol and discharged one month after surgery. While unequivocal proof that the gall bladder was the source of this infection is lacking, it does appear to be a valid assumption that the chronic cholecystitis was in part caused by the typhoid bacilli and that during the operation bacterial invasion of the blood stream took place.

Case 7. In the past year we have had the opportunity to observe a few cases of cerebral ventriculitis of unusual etiology. One case involved a one year old male who was admitted to the hospital for treatment of an internal hydrocephalus which had developed from a previous attack of meningitis. On admission the spinal fluid was cytologically and biochemically within normal limits and was sterile. Burr hole openings were made in the skull to relieve the internal cerebral pressure and to examine

the lateral ventricles. A purulent ventricular fluid was obtained from the right and left lateral ventricles, which gave pure cultures of *Escherichia coli*. The spinal fluid was still sterile indicating an obstruction of the fourth ventricle and localization of the infection. An extensive course of neomycin therapy was instituted and after 20 days of treatment the ventricular fluid was sterile.

Case 8. A second case was that of a 28 year old female who was admitted 10 months after surgical removal of an acoustic neurinoma. At the time of admission the patient complained of severe headaches and frequent vomiting spells. Her temperature was elevated and her neck was rigid. Surgical cannulation of the lateral ventricles yielded a copious amount of a purulent exudate. Cultures from this exudate proved to be *Diplococcus pneumoniae*. Prompt treatment with penicillin cleared the ventricles of infection and recovery has been slow but progressive.

In both of these cases the prompt identification of the responsible organisms and the determination of antibiotic sensitivity was of great aid in preventing extensive tissue destruction.

It is our contention that each one of these cases clearly indicates the need for more than haphazard microbiology. It is also our belief that acceptable microbiology is within the scope of every clinical laboratory. Of course, many laboratories are currently equipped and staffed to perform acceptable bacteriology. Unfortunately, however, the majority of laboratories is either poorly equipped or understaffed and substandard bacteriology is the result. In an attempt to alleviate this situation where it exists, we would like to propose an outline of routine inoculation procedures which can be adapted to any laboratory. These procedures are flexible and if properly executed will result in a dependable microbiological service. This outline is not intended to replace common sense and is therefore subject to modifications when conditions so indicate. Finally, this outline is intended to serve as an initial guide to insure growth of the organisms present in the specimens submitted. Once growth is obtained, then definitive identification of the organisms is in order by employing appropriate biochemical and serological tests. (Moss, 1947; Hepler, 1951; Edwards and Ewing, 1951; Haley, 1952; Kalz and Murray, 1952.)

1. Any material submitted to the laboratory for culture should also be smeared on a slide and a gram-stained preparation should be made even though it has not been specifically requested. When culture for acid-fast bacilli is requested, then an acid-fast stained preparation is also necessary.

2. Cultures
   a. Blood

Strict aseptic technique is essential. Ten ml of blood should be added to a bottle containing 50 ml of sterile blood culture broth. When a blood culture for brucellosis or tularemia is requested, blood culture bottles containing carbon dioxide* or the Castenada bottle utilizing a solid and a liquid medium (Castenada, 1947; Marvin, 1949) should be used.

b. Sputum

Routine cultures should be streaked on a blood agar plate and inoculated into a tube of thioglycollate broth; if fungus culture is requested or if fungal structures are observed on stained preparations, inoculate tubes of Littman's medium and Sabouraud's medium and incubate at room temperature. In culturing for tubercle bacilli it is advisable that the sputum be digested and concentrated prior to inoculation in order to minimize overgrowth of other organisms. The sputum concentrate should be inoculated on Petragnani and Lowenstein-Jensen media.

c. Throat and naso-pharynx

The swabs should be streaked on a blood agar plate and inoculated into a tube of thioglycollate broth. If culture for diphtheria bacilli is requested or indicated, inoculate tubes of Loeffler's coagulated serum medium and chocolate tellurite agar.

d. Cerebro-spinal and ventricular fluid

The fluid should be first examined for the presence of a pellicle and if one is present, it should be carefully removed, placed on a clean slide and appropriately stained. The remainder of the fluid should be centrifuged for at least 30 minutes at a high speed (2000-2500 rpm), the supernatant fluid decanted and the sediment inoculated on a blood agar plate and a chocolate agar plate. Chocolate agar plates should be placed in a CO_2 jar prior to incubation. Petragnani and Lowenstein-Jensen media should be inoculated when cultures for tubercle bacilli are indicated.

e. Transudates, exudates, ascitic fluid, pleural fluid, joint fluid, etc.

The material should be routinely inoculated on a blood agar plate and into a tube of thioglycollate broth. Cultures for the gonococcus should be streaked on a chocolate agar plate; for acid-fast bacilli inoculate the prescribed media.

f. Abscesses, ulcers, wounds, surgical swabs, etc.

The submitted material should be routinely inoculated on a blood agar plate and into a tube of thioglycollate broth. If anaerobic organisms are suspected, an additional tube of thioglycollate broth should be placed in a boiling water bath for 10 minutes, cooled and then inoculated.

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* These bottles are available commercially and can be purchased from Becton-Dickinson Co., Rutherford, N. J.

g. Urine

The urinary sediment obtained by centrifugation should be streaked on a blood agar plate and a plate of eosin methylene blue agar or MacConkey's agar. If a gonorrheal infection is suspected, the sediment should be inoculated on a plate of chocolate agar. If a culture for gram-negative enteric bacilli is requested, proceed as outlined for stool cultures.

h. Feces

The stool specimen should be streaked directly on plates of bismuth sulfite agar, desoxylcholate-citrate agar and MacConkey's agar. A small portion of the specimen is thoroughly emulsified in a tube of selenite broth. After 18-20 hours incubation, the growth in selenite broth is subcultured on plates of *Salmonella-Shigella* agar and bismuth sulfite agar. Cultures from suspicious colonies on any of the agar plates are made on Russell's double sugar, and checked for motility, and for production of H_2S, indole and urea. If the initial biochemical reactions suggest an enteric pathogen, additional sugar broths are inoculated and slide agglutination tests are performed with *Salmonella* and *Shigella* antisera. (Kauffmann and Edwards, 1947; Kauffmann, 1950; Edwards and Ewing, 1951; Edwards and Kauffmann, 1952.)

i. Fungus cultures

Any material submitted to the laboratory for fungus culture should first be examined microscopically by preparing wet mounts in 10 per cent KOH and in cotton blue. Cultures are made by inoculating tubes of Littman's agar, Sabouraud's agar and a blood agar plate. If a culture for *Actinomycetes* is requested or indicated, a tube of thioglycollate broth containing 3-4 drops of normal plasma or serum should be inoculated in addition to the above media.

In conclusion, it is our belief that many clinical laboratories are following limited and ill-defined routine procedures for microbiological work. We have presented a few cases of infectious diseases which would have been missed if such limited procedures had been adhered to. We have presented a brief outline of practical microbiology which can be adopted by any clinical laboratory to render a satisfactory microbiological service.

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ANTISTREPTOLYSIN-O TITRES IN ALLERGIC PURPURA AND RELATED DISEASES*

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Antistreptolysin was first demonstrated by Todd[1] in patients recovering from acute streptococcal infections, and also in those with rheumatic fever and glomerulo-nephritis. This work has since been confirmed by many workers.

Streptococci of Lancefield's groups A, C, and G, the three organisms which cause the majority of human streptococcal infections, are now known to produce two distinct hemolysins, the oxygen-labile Streptolysin "O" and also Streptolysin "S." Approximately 85% of clinical infections due to streptococci of Groups A, C, and G result in the production of antistreptolysin "O" during the second to third week of convalescence.[2] It has also been observed that in patients with rheumatic fever and acute glomerulo-nephritis the titres were significantly higher than in those who had an uncomplicated streptococcal infection.[3, 4]

Since the streptococcal infection preceding the onset of rheumatic fever, acute glomerulo-nephritis and other allergic conditions may be so mild as to pass unnoticed, the etiological significance of the antistreptolysin test in these conditions is obvious.

During a recent scarlet fever epidemic we have encountered a number of cases of seemingly unrelated allergic purpuras and thought it of interest to ascertain the antistreptolysin titres of the patients in order to help establish the etiological role of the hemolytic streptococcus. In the course of this investigation antistreptolysin titres were also determined in a case of purpura fulminans, three cases of erythema nodosum, and some cases of rheumatic fever and glomerulo-nephritis.

Methods:

Preliminary antistreptolysin "O" tests were run on two groups of normal controls, whose ages ranged between 44-57 and 18-25. The titres in these age groups averaged 52 and 90 units respectively, with a maximum of 100 units in each group. The serum from one of the rheumatic fever patients with a titre higher than 1,000 units was used as a positive control, and a serum with a titre of 50 units was used as a negative control throughout the tests.

The method used for performing the tests was that described by Rantz and Randall[5]: Varying dilutions of the patient's serum are mixed with a standardized amount of reduced streptolysin (antigen) and incubated for a prescribed period. During this time, the lytic activity of the antigen is neutralized by such antistreptolysin as may be present in the serum. If the standardized

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amount of antigen (streptolysin) is in excess of the amount of antistreptolysin present in the serum, subsequent addition of a suspension of red cells (indicator) will result in haemolysis. Conversely, if the antibody (antistreptolysin "O") is in excess, there will be no lysis of the red cells.

Streptolysin "O" antigen is composed of the standardized, bacteria free, partially purified proteins recovered from the meat broth in which Group A streptococci have actively grown. When freshly prepared, this is in the oxidized state, and only becomes lytic when converted to the reduced state. This is accomplished by the presence of M/25 cysteine monohydrochloride. Standardized amounts of antigen* are supplied in the desiccated, oxidized state, together with an alkaline buffer, and the required amount of cysteine to convert it to the reduced state upon addition of a specified amount of water. Two sizes are supplied; the smaller vials are to be rehydrated by the addition of 10 ml. of water, and furnish sufficient antigen for 2 tests. The larger vial requires the addition of 25 ml. water, and is sufficient for six tests. After the water is added to the vial, the reagent is allowed to "age" for 10 minutes at room temperature. The active (reduced) streptolysin "O" thus prepared is stable for only 2 hours. It was the practice, in these investigations, to use the reagent immediately the 10 minutes required for "aging" was up, thus giving a standardized timing to all the tests. This, of course, required that the serum dilutions were all prepared and ready for the addition of the antigen as soon as the ten minute aging period was completed.

Technique:

The serum to be tested should be freshly drawn. Where it was not possible to perform the test the same day, the serum was stored in the freezing compartment of the refrigerator. Before use the serum was inactivated at 56° C. for 30 minutes. Primary dilutions of serum were made of 1:10, 1:100, and 1:500 using normal physiological saline as the diluent. These were prepared as follows:

A. (1:10 dilution): 0.3 ml. serum with 2.7 ml. saline.
B. (1:100 dilution): 0.5 ml. solution A with 4.5 ml. saline.
C. (1:500 dilution): 1.0 ml. solution B with 4.0 ml. saline.

These dilutions were made in Wasserman test tubes (13 x 100 mm.). For the actual test mixtures, serological tubes 11 mm. x 70 mm. were used.

Using 1 ml. serological pipettes for the addition of the serum, and a 10 ml. serological pipette for bringing the serum volumes up to 1.0 ml. with saline, tubes were set up as below (Table I). 10 ml. serological pipettes were used for the addition of the lysin and cell suspension.

* The product used in these investigations is supplied by the Bacto. Company (Bacto-Streptolysin O reagent).

TABLE I

	SERUM DILUTION			Control
	1:10	1:100	1:500	Cells Lysin
1	0.8 0.2	1.0 0.8 0.6 0.4 0.3	1.0 0.8 0.6 0.4 0.2	0 0
2	0.2 0.8	0 0.2 0.4 0.6 0.7	0 0.2 0.4 0.6 0.8	1.5 1.0
3	0.5 0.5	0.5 0.5 0.5 0.5 0.5	0.5 0.5 0.5 0.5 0.5	0 0.5
	Incubate Tubes for 15 Minutes at 37° C.			
4	0.5 0.5	0.5 0.5 0.5 0.5 0.5	0.5 0.5 0.5 0.5 0.5	0.5 0.5
	Incubate Tubes for 45 Minutes at 37° C.			
5	12 50	100 125 166 250 333	500 625 833 1250 2500	

1—ml. diluted serum. 2—ml. saline. 3—ml. reduced lysin. 4—ml. 5% R.B.C. suspension. 5—Unit value of tube.

In place of the rabbit cells used in the original test of Rantz and Randall,[5] human Group O cells from the blood bank were found to be satisfactory and were used throughout these tests. The cells were washed with normal saline (0.85 per cent NaCl) and centrifuged for 5 minutes at 2000 r.p.m. The red-tinged supernatant was siphoned off and the process repeated (usually three times) until a clear colorless supernatant was obtained. A 5 per cent suspension of cells was prepared by adding 1 ml. of the washed, packed cells to 19 ml. saline.

The reduced lysin was prepared as described above, and after the required aging time (10 minutes) was completed, 0.5 ml. was added to all the tubes containing diluted serum, and to the lysin control tube (see Table I). The tubes were shaken well, and placed in the incubator at 37° C. for 15 minutes, to allow reaction of lysin and antibody (antistreptolysin "O").

After incubation, 0.5 ml. of the 5% cell suspension, prepared as above, was added to all the tubes (see Table I). The tubes were shaken separately by hand to insure a uniform suspension. All the tubes containing a given serum were shaken thus, before proceeding with the addition of cells to the next serum under test. The racks of tubes were then re-incubated at 37° C. for 45 minutes, the tubes being again shaken individually at the end of the first 15 minutes of this period.

After the 45 minutes incubation, the tubes were centrifuged at moderate speed for 1½ minutes (the speed and time were kept constant for all tubes and throughout all the tests). The supernatant liquid was examined for traces of haemolysis. The highest dilution of serum that completely inhibited haemolysis was taken as the titre of the serum. Using the aforementioned reagent, the end points were usually clearly marked but one or two doubtful tubes were examined spectroscopically for the hemoglobin absorption bands. (These are easily detected by first examining the lysin control, in which the cells are completely

haemolyzed.*) The unit value of the serum was expressed in terms of Todd units. This is the reciprocal of the volume of serum (ml.) necessary to neutralize a standard amount of streptolysin "O" of known potency. This standard amount of streptolysin "O" is known as a combining unit, and the commercial preparation used in these tests contains 1 combining unit in 0.5 ml. The titre (in Todd units) is the reciprocal of the dilution of serum that combines with, and just neutralizes one combining unit, so that there is no hemolysis in that tube. (See Table II for example.)

TABLE II

TUBE	1	2	3	4	5	6	7
Volume of undiluted serum (ml.)	0.08	0.02	0.01	0.008	0.006	0.004	0.003
Volume of diluent (saline)	0.2	0.8	0.0	0.2	0.4	0.6	0.7
Final dilution of serum (after addition of 0.5 ml. lysin)	1/12.5	1/50	1/100	1/125	1/166	1/250	1/333
Reciprocal of serum dilution (= unit value in Todd units)	12.5	50	100	125	166	250	333
Haemolysis	None	None	None	Trace	1+	2+	Complete

The antistreptolysin titre in this example would be 100 Todd units.

Interpretation:

The pathogenic hemolytic streptococci are the only organisms known to produce Streptolysin "O," and the antistreptolysin formed against this antigen appears to be specific.[2] While a slightly raised antistreptolysin titre may only reflect past infection, a rise in titre, or a high titre per se is taken to be indicative of active streptococcal disease.

On an average the normal titre appears to be higher in younger persons, the maximum levels being found in the 8-12 year age group, while low titres (50 units or less) are the rule in persons of the 40-60 age group.[6]

Case Histories and Laboratory Findings:

Case 1 (S.L.) Allergic purpura

This two year old boy was admitted to hospital on Jan. 28th, 1953. A week previously he had had a mild sore throat, but no physician was called. On admission he had a temperature of 102° F. and there were several erythematous areas on the legs. The child was listless, but not acutely ill. The following day the temperature rose to 103° and the patient appeared ill. The dorsum of both feet became edematous, with a number of ecchymoses

* In tests performed subsequently to this series, and using a later batch of Bacto Streptolysin "O" reagent, it has been found entirely unnecessary to use the spectroscope, since none of the tests failed to give a clear cut end-point when the tubes were viewed against a white background.

extending up the side of the left leg. Similar areas appeared on the left hand.

The W.B.C. on admission was 12,600 per cu. mm., with an absolute increase in polymorphonuclear leucocytes. There was no anaemia and the platelets were normal. The bleeding time, clotting time and prothrombin time were normal. The following day the W.B.C. had risen to 15,750 per cu. mm. and the polymorphs showed marked toxic granulation.

The urine was negative, and a throat culture revealed no pathogens. The antistreptolysin "O" titre two days after admission was 250 units, and after one week it had risen to over 2,500 units.

The patient received Penicillin therapy and made an uneventful recovery. He was discharged on Feb. 8th, 1953.

Case 2 (R.D.) Allergic purpura

A five year old girl was admitted to hospital on Feb. 4th, 1953, with a sore throat and a number of bruises. The lower limbs showed widespread erythematous areas and some purpuric spots and echymoses, and there were also some erythematous areas over the upper extremities. The lesions were particularly marked over the extensor surfaces of the extremities, but were present over the whole circumference of the thighs. There was no associated temperature, and the patient was not considered ill.

The hemoglobin, W.B.C. and sedimentation rate were normal, as were also the bleeding time, clotting time, platelet count, prothrombin time and fibrinogen concentration.

Urinalysis was essentially negative. A heavy growth of hemolytic streptococci (Lancefield group A) was obtained from a throat swab. The organism was sensitive to Penicillin in vitro. An antistreptolysin "O" test was performed the day after admission and showed a titre of 50 units. Eighteen days later the titre had risen to 625 units.

The patient was treated with Vitamin C, Vitamin K and Pyribenzamine and later with Penicillin. She made an uneventful recovery, and was discharged on Feb. 12th, 1953.

Case 3 (B.G.) Allergic purpura (Henoch)

This two year old boy had been unwell for a month or so before hospitalization, suffering from repeated colds and associated mild sore throat. Four days prior to admission, a fine, macular rash appeared over the limbs. This faded, and four days later a purpuric rash appeared over the entire body with the exception of the face. He also seemed to suffer from colicky abdominal pains; these symptoms occasioned his admission to hospital on Feb. 1st, 1953.

The hemoglobin was 67% (9.9 gms.) with a C.I. of 0.88. The W.B.C. was 13,500 per cu. mm. with 41% polymorphs and 51%

lymphocytes. The platelet count, bleeding time, clotting time and prothrombin time were normal. The urinalysis was essentially negative, but occult blood was present in the stools. Throat culture yielded a scanty growth of hemolytic streptococci (Lancefield Group A).

The patient was acutely ill for about three weeks, with a swinging temperature. Several fresh crops of erythema and purpura developed at approximately 2-3 day intervals. The face, hands and feet were edematous. During this period the W.B.C. rose to 20,900 per cu. mm. and there was an absolute increase in polymorphs. The hemoglobin fell to 56% (8.3 gms.) while the urinalysis now showed + + + albumin, large numbers of R.B.C.'s, 2-5 W.B.C.'s and some granular and hyaline casts were H.P.F. Occasionally, frank hematuria was noted. Two days after admission the antistreptolysin "O" test revealed a titre greater than 2,500, and a week later it was shown to be 8,333 units.

Treatment consisted of the administration of Cortisone, Penicillin and one blood transfusion. The patient is at present making a slow recovery.

Case 4 (Y.S) Allergic purpura

This four year old girl was admitted to hospital on Dec. 28th, 1952, with a temperature of 102.3° F. There were maculo-papular areas on the arms, legs and buttocks, and a thick encrusted lesion was present on the chin. The extremities showed a number of purpuric and ecchymotic areas, and there was marked edema around both orbits. An infected, crusty lesion was present over the leg. The tongue was edematous and heavily coated. The patient was considered fairly ill for a few days.

The W.B.C. was 24,750 per cu. mm. and the polymorphs showed toxic granulation. The hemoglobin was normal and the platelets were adequate. The sedimentation rate was 44.0 mm. in 45 minutes (Westergren). The bleeding time, clotting time and the fibrinogen level were normal, and the prothrombin time was only slightly prolonged. On the seventh and ninth days after admission the urinalysis showed occasional W.B.C.'s and R.B.C.'s per H.P.F. but otherwise the urinalysis was negative.

A culture was made of scrapings from the tongue, and also from the lesion of the leg. A heavy growth of hemolytic streptococci (Lancefield Group A) was obtained in each case. The organism was sensitive to Penicillin in vitro. The antistreptolysin "O" titre was 12 units on admission, and one week later it had risen to 125 units. A month afterwards the titre had not changed further.

The patient's temperature remained high for a few days, but returned to normal soon after the administration of Penicillin. The lesions healed rapidly and throat cultures became negative. The patient was discharged on Jan. 22nd, 1953.

Case 5 (J.V.) Allergic purpura (Schoenlein)

This fifty-five year old lady was admitted to hospital on Oct. 4, 1952, complaining of painful joints in the left elbow region and around the knees. The arms showed erythematous areas and the legs were covered with petechial hemorrhages. Her temperature was 100.2° F. and the patient appeared fairly ill for a few days.

Her previous history was entirely negative and there was no evidence of a sore throat or scarlet fever.

There was no anaemia or leucocytosis. The bleeding and clotting times were normal, and the prothrombin time was only slightly prolonged. The platelet count was 664,000 per cu. mm. The sedimentation rate was elevated to 40 mm. in 45 minutes (Westergren) and later rose to 63 mm. There was a slightly elevated eosinophil count, and a sternal puncture revealed a distinct marrow eosinophilia. An Old Tuberculin test gave no reaction and the urine showed no abnormalities. The antistreptolysin "O" titre was 625 units two days after admission. It was not repeated.

The patient made a slow but uneventful recovery and was discharged on Oct. 28th, 1952.

Case 6 (G.L.B.) Purpura fulminans

This boy, aged three years, was admitted to hospital on Feb. 27th, 1951, with a severe hemorrhagic diathesis. The previous history is somewhat vague, but there was evidence of a sore throat within one week prior to the onset of the purpuric symptoms. In addition to epistaxis, conjunctival bleeding, haematomata in both eyes, and haematuria, there were bluish-black discolorations over large areas of both legs, the right arm, the left forearm and the right cheek. The hemorrhagic areas were well defined and surrounded by edema. Many vesicles had formed over the affected areas and contained a clear or sanguinous fluid.

There was a moderate anemia and thrombocytopenia, and a marked polymorphonuclear leucocytosis. The blood urea was slightly raised (77 mg.%). The bleeding time was slightly prolonged, and the blood was incoagulable, owing to the complete absence of fibrinogen. A throat swab yielded a growth of E. Coli. The antistreptolysin "O" titre was higher than 1,000 units.

The child was seriously ill for a number of weeks. Treatment consisted of a simple transfusion, an attempted exchange transfusion, administration of fibrinogen and antibiotic therapy. The lower extremities meanwhile had become gangrenous and had to be amputated. After this complete recovery took place, except for scars over the other affected areas. The patient was discharged on Aug. 21st, 1951.

Case 7 (E.N.) Erythema nodosum

This fourteen year old girl had had a sore throat three weeks prior to hospitalization. Three days before admission she devel-

oped painful erythematous nodules over the extensor surfaces of both legs. She was admitted to hospital on Feb. 6th, 1953, but was not considered very ill.

The laboratory findings were essentially negative. The antistreptolysin "O" titre on admission was 1,250 units, and twelve days later it was greater than 2,500 units. An Old Tuberculin test was negative. The patient made an uneventful recovery and was discharged on Feb. 21st, 1953.

Case 8 (P.B.) Erythema nodosum

This young married woman aged eighteen had had a sore throat about one week prior to admission. Subsequently she developed a "rash" over the legs, which exhibited painful, erythematous nodules. The laboratory findings were negative except for a slightly raised sedimentation rate. The antistreptolysin "O" titre on admission was 1,250 units. The test could not be repeated. The patient was discharged after an uneventful recovery.

Case 9 (M.K.) Erythema nodosum

This was also a young married woman, nineteen years of age. She had had a severe sore throat one month prior to hospitalization. This was followed after one week by the appearance on her legs of lesions characteristic of erythema nodosum. At first she was kept at home, but as the lesions persisted she was admitted to hospital.

The laboratory findings were essentially negative, but the sedimentation rate was 36 mm. in 45 minutes (Westergren) and a throat culture yielded a moderate growth of hemolytic streptococci (Lancefield Group A). The antistreptolysin "O" titre on admission, and twelve days later, was greater than 2,500 units.

Treatment consisted of bed rest and the administration of salicylates. The patient is making an uneventful recovery.

Cases 10-13

This group comprises three typical cases of rheumatic fever

TABLE III

Case No.	Age of Patient (Years)	Diagnosis	First Titre (Todd Units)	Second Titre (Todd Units)
1.	2	Allergic purpura	250	> 2,500
2.	5	Allergic purpura	50	625
3.	4	Allergic purpura	> 2,500	8,333
4.	4	Allergic purpura	12	125
5.	55	Allergic purpura	625	.....
6.	3	Purpura fulminans	1,250	.....
7.	14	Erythema nodosum	1,250	> 2,500
8.	18	Erythema nodosum	1,250	.....
9.	19	Erythema nodosum	> 2,500	> 2,500
10.	17	Rheumatic fever	> 2,500	.....
11.	14	Rheumatic fever	1,250	.....
12.	14	Rheumatic fever	500	.....
13.	50	Ac. Glomerulo-nephritis	250	.....

and one of acute glomerulo-nephritis. They require no further comment. (See Table III.)

Discussion:

The allergic purpuras comprise a group of non-thrombocytopenic purpuras, which are characterized by purpura in association with one or more of the common symptoms of allergy. They include not only well defined entities such as Henoch's and Schoenlein's purpuras, in which the lesions are associated with gastro-intestinal and joint symptoms respectively, but also purpuras associated with erythema, urticaria or effusions of serum into subcutaneous or submucous tissues or viscera.

The cause of the purpura is assumed to be an increased capillary permeability with exudation of plasma and blood cells. The blood vessels in the neighborhood of the hemorrhages may appear entirely normal, they may show a surrounding inflammation, or there may be a necrotising inflammation of the vessels themselves.[6]

These pathological findings explain the diversity of symptoms that may be encountered. The true nature of these purpuras is as yet not fully understood, and in only a minority can an allergen be demonstrated. The first five cases in this paper represent examples of such allergic purpuras.

The antistreptolysin titres are summarised in Table III. The first three cases show such a large rise in titre that recent streptococcal infection must be implicated. Case No. 4 shows a final titre only slightly higher than the highest normal control (100 units), but the 3-tube rise in titre is nevertheless significant.[8, 9]

The case of Schoenlein's purpura (No. 5) did not occur during the streptococcal epidemic, and unfortunately a second antistreptolysin test was not carried out. However, the titre of 625 units in a person aged fifty-five would appear to imply the etiological role of the hemolytic streptococcus, in spite of the entirely negative clinical history.

Purpura fulminans was first described by Henoch in 1886. Its characteristic features are a rapid, symmetrical spread of hemorrhages into the skin of the extremities, with a tendency to necrosis and vesicle formation. The disease is almost invariably fatal, and is regarded by most authors as an extreme degree of allergic purpura. The cutaneous lesions are possibly related to the Shwartzman or Arthus phenomenon. These cases are extremely rare, but many of those reported in the literature have followed scarlet fever after a latent period of 2-4 weeks. The remaining cases recorded in the literature are of uncertain etiology. In the case reported here (No. 6) the antistreptolysin titre would appear to establish the etiological significance of the hemolytic streptococcus. This could not be substantiated by bacteriological means,

since the patient had received antibiotic therapy prior to hospitalization.

Erthema nodosum is characterized by fever, malaise and painful erythematous areas on the skin of the extremities. This syndrome may occur in a multitude of conditions, but is seen with particular frequency in cases of streptococcal infections and early pulmonary tuberculosis. It is considered to be a non-specific syndrome caused by an immune response to an infection or drug. The cases reported here[7, 8, 9] were all admitted to hospital during the height of the streptococcal epidemic, and each presented a history of sore throat. These features suggested the streptococcal origin of the lesions, and although only one of the throat cultures was positive, this theory was substantiated by the high antistreptolysin titres found in each case.

Cases 10 to 13 confirm the expected high titres in rheumatic fever and acute glomerulo-nephritis. It is of interest to note here that the lowest titre was encountered in an older person (Case 13).

It is also evident from Table III that, except for No. 4, all of the cases showed titres that were considerably higher than those usually encountered in uncomplicated streptococcal infections.[11] Titres approaching this magnitude have been noted consistently in rheumatic fever and acute glomerulo-nephritis, and it is of great interest to note the occurrence of equal, and even higher titres in the other allergic conditions.

SUMMARY

Six cases of allergic purpura and three cases of erythema nodosum have been presented. The significance of the antistreptolysin "O" titres observed in these patients has been discussed.

ACKNOWLEDGMENTS

I would like to thank Dr. G. Kent, pathologist, for his helpful criticism and encouragement, and the doctors in charge for allowing me to present their cases in this paper. My thanks are also due to Sister Mary Cornelia R.T. and Miss L. Smith R.T. for their kind co-operation and help with some of the laboratory procedures.

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PROTHROMBIN TIME*

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Normally a clot, or thrombus, is formed to stop or prevent bleeding from a damaged blood vessel. However, there are conditions in which the blood clots within the cardiovascular system. When this occurs, tissue or organs are damaged because their blood supply is partially or completely blocked. Examples of this type of clot formation are thrombosis of the coronary arteries of the heart and the blood vessels of the brain. Besides damage to the tissue as a result of interference of its blood supply, thrombi are dangerous because a portion of this clot may break off and be carried in the blood stream to another part of the body. These are emboli and may result in damage to certain tissues where they lodge and may even cause sudden death when a large thrombus in a vein breaks loose and is carried to the lung where it blocks the pulmonary artery. An example is a thrombophlebitis of the leg with a complicating pulmonary embolism.

The use of anticoagulant drugs, such as dicumarol, has proved helpful in the treatment of venous and arterial thrombosis and congestive heart failure, and in the thromboembolic complications of coronary thrombosis. The safe administration of these drugs depends entirely upon reliable determinations of the prothrombin time in order that the dose of the drug can be controlled. The accuracy of the results are almost as important as correct blood typing. For unless the doctor can rely on the results of the prothrombin determination to control the dose, a patient may bleed to death from receiving too much of the drug, or the patient may die from further extension of the thrombus from not receiving enough of the drug to reach a therapeutic level. Thus, the necessity of accurate determinations of the prothrombin time is evident, and this can only be accomplished by fully understanding the technique and its pitfalls.

The coagulation of blood is an extremely complex process in which numerous constituents of the blood play a role. The *platelets* do not contain thromboplastin, but upon rupturing furnish an activator, thromboplastinogenase. This activates the thromboplastinogen, found normally in plasma, to thromboplastin. In hemophilic blood there is a lack of thromboplastinogen in the plasma; while in thrombocytopenic purpura there is a decrease in the amount of thromboplastinogenase due to a decrease in platelets. Another source of thromboplastin is juice from injured

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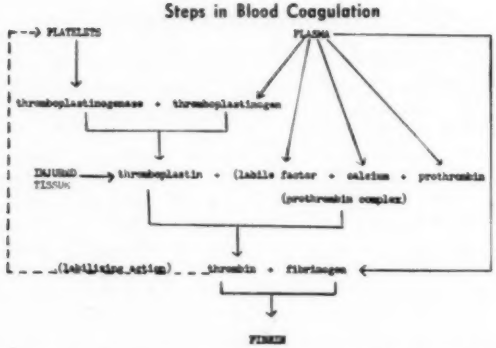

tissue. It is very important that a clean puncture of a vein be made in collecting the blood so that the blood will not be contaminated with tissue juice. If difficulty in puncturing the vessel is encountered, a few ml. of blood should be withdrawn after the vein is entered and then the syringe exchanged for a clean one. The blood withdrawn in this syringe is used for the test. Theoretically this should always be done, but it is not practical and not necessary if there is no difficulty in finding the vein. In taking the sample of blood for the test the anticoagulant should not be placed in the syringe for two reasons, (1) the possibility of injecting some of the solution into the patient, and (2) it is difficult to always withdraw an exact amount of blood. The importance of having the correct proportion of anticoagulant to blood will be discussed later.

Prothrombin is a plasma protein formed in the liver from vitamin K. Therefore, a prolonged prothrombin time may be found in severe liver damage or in vitamin K deficiency. The latter may be due to (1) a dietary deficiency, (2) lack of formation of vitamin K by the bacteria of the intestine due to sterilization of the intestinal tract with antibiotics, such as aureomycin, and (3) lack of absorption of vitamin K due to the absence of bile in the intestine in obstructive jaundice. It is extremely important to have a control prothrombin determination on each patient before anticoagulant therapy is started in order to rule out a decrease in prothrombin due to these factors. To avoid confusion the word control prothrombin will refer to the control on each patient before therapy is started, while normal control will refer to the normal plasma used each day to check the solutions.

Dicumarol reduces the formation of prothrombin in the liver; it is a true anti-vitamin K. When prothrombin has been decreased to a dangerous level from the administration of too much dicumarol, vitamin K therapy will increase the formation of prothrombin. The reduction of prothrombin by dicumarol varies with the dose. Patients

also differ greatly in their sensitivity to the effect of this drug, therefore, the dose must be carefully controlled with prothrombin times. With a clinically effective dose, the prothrombin time does not return to normal for 2 to 10 days after the last dose. Some of the newer drugs, such as Tromexan, give erratic results for prothrombin time. A small dose may give a greatly prolonged prothrombin time, while a large dose may not show any appreciable change in prothrombin.

Salicylates act in the same manner as dicumarol but to a much less extent. This is exaggerated by a dietary deficiency of vitamin K. A prolonged prothrombin time for the control test before the administration of dicumarol may be explained by salicylate therapy. Salicylates may also reduce the platelets.

The anticoagulant action of heparin is complex and prevents clotting in three different ways: (1) It prevents the conversion of prothrombin to thrombin, therefore, it is an anti-prothrombin; (2) with a co-factor in plasma it inactivates thrombin practically instantaneously, and (3) by removing thrombin it prevents the disintegration of platelets. All these effects are measured by Lee and White coagulation time which is used to control heparin therapy. However, because heparin does act as an anti-prothrombin, it affects the prothrombin time. Therefore, blood for prothrombin determinations must *not* be obtained during heparin therapy. It is advisable to insist that the control prothrombin test be done before any type of therapy is started. Heparin is sometimes given during the first two days of dicumarol therapy because it has an immediate effect on the clotting mechanism while dicumarol does not reach its maximum effect for 48 hours. There are 2 types of heparin, the effects of one injected intramuscularly last for 8 to 12 hours and of the other injected intravenously last only 4 hours.

Quick[1] believes that prothrombin exists in the plasma in two forms; as active prothrombin which is measured in the one-stage method and prothrombinogen, a precursor of prothrombin. The latter is converted to prothrombin when plasma comes in contact with a rough surface such as etched glassware. The two-stage method for the determination of prothrombin converts the two types of prothrombin to thrombin which is measured by its ability to clot a standard fibrinogen solution. This method is too time-consuming to be used in a busy routine hospital laboratory. The shorter one-stage method yields results adequate for the control of anticoagulant therapy.

A factor present in plasma designated by Quick[1] as the *labile factor* is essential for the formation of thrombin. Other investigators have applied the following terms to this factor: Factor V, accelerator factor, thromboplastin co-factor, and ac-globulin. This factor diminishes in stored plasma and is affected by heat. Its instability is greatly increased in oxalated plasma due to the absence of calcium ions. Centrifugation of blood at high speed and for long lengths of

time destroys this factor. For these reasons the blood must have a short, light centrifugation, then placed in the refrigerator, and the prothrombin time determined as soon as possible. The labile factor is not affected by dicumarol or by vitamin K deficiency. Clinically it is rare to find a case with a diminished labile factor. There are only a few cases reported in the literature. Severe liver damage may reduce the labile factor, but in these cases the prothrombin is also reduced.

The role *calcium* plays in coagulation is unknown, but it is necessary for the formation of thrombin. Only the bound calcium takes part in this reaction. Calcium is removed from the blood by the addition of either sodium oxalate or sodium citrate as anticoagulants. Therefore, calcium must be added in the prothrombin test for the formation of a clot. When calcium chloride is added in performing the test, it immediately interacts with any excess of oxalate or citrate present. A relative excess of either of these will, therefore, exert an influence upon the required concentration of calcium. The amount of calcium is very important because as the prothrombin decreases, the calcium concentration needed for maximum activity is increased. If some of the calcium added reacts with an excess of oxalate or citrate, there may not be enough left for all the prothrombin to be changed to thrombin and a prolonged prothrombin time will be obtained. Thus the importance of accurate measurement of the sodium oxalate or citrate and calcium chloride solutions as well as having the correct concentration of these solutions is evident. Quick[1] has found that 0.02 M solution is preferable to 0.025 M which he used in his original method. This concentration is low enough to have only a slight inhibiting effect on the reaction, but still high enough to give maximum activity when the prothrombin time is prolonged. In his research he uses both a 0.01 M and a 0.02 M solution of calcium chloride.

Prothrombin plus the labile factor and calcium form a prothrombin complex which reacts with thromboplastin to form thrombin. A diminution of any constituent of the prothrombin complex will result in a lowered production of thrombin and thus a prolonged prothrombin time.

As thrombin is formed it converts by enzymatic action the fibrinogen in the plasma to fibrin, which is the clot. A decrease in fibrinogen in the plasma has little effect on the prothrombin time. Thrombin also acts directly on platelets as soon as it is formed and accelerates their agglutination and disintegration, thus yielding more thromboplastinogenase to activate thromboplastinogen which in turn acts to form more thrombin. This cycle results in a marked acceleration of the clotting mechanism in the body. However, as fibrin is formed, it absorbs thrombin and thus keeps the clotting mechanism under control.

Four substances are necessary to perform a prothrombin determination: an anticoagulant, plasma, thromboplastin, and calcium chloride solution.

The anticoagulant generally preferred is a 0.1 M solution of

sodium oxalate. When a 0.1 M solution of sodium citrate is used, the labile factor disappears more slowly, but the conversion of prothrombinogen to prothrombin is accelerated. Consequently a normal prothrombin time is usually one second shorter when sodium citrate is used than when sodium oxalate is used. When sodium citrate is employed the test must be done within 2 hours after the blood is collected, while oxalated blood will keep 3 hours. We have had better success using sodium oxalate than sodium citrate. The solution should be made in small enough quantities that it is prepared once a month. It should be kept in the refrigerator when not in use. As mentioned before it is necessary to have the correct proportion of oxalate to blood. We etch our test tubes used for collecting blood at 2 ml. or 5 ml. depending upon the amount of blood desired. Some patients are difficult to bleed and it is easier to obtain 2 ml. of blood than 5 ml. Either 0.2 ml. or 0.5 ml. of oxalate solution is pipetted into the tubes the day before they are to be used and then placed in the refrigerator so they will be cold the next morning. We prefer etching our own centrifuge tubes instead of using graduated ones because it is much easier to fill the tube to a single mark circling the tube. The graduations on a graduated tube are so close together, it is difficult to see the meniscus of the blood and also to quickly pick out the correct mark. The etching on the tubes makes an easy identification for keeping these tubes separated for prothrombin determinations.

The labile factor deteriorates when blood is kept at room temperature. In order to keep the bloods cold during the collection period, Quick devised a simple and unique portable ice container. An empty soup can is placed in the center of an empty pound coffee can, the coffee can is filled with water and placed in a deep freeze so the water is completely frozen. This ice container is taken to the floors while collecting the bloods. The tubes of blood are placed in the soup can and thus kept cold. This is particularly useful in hot summer weather. We have a rule that all bloods for prothrombin determinations must be centrifuged within 30 minutes after the blood is drawn and the plasma separated and placed in the refrigerator. When a number of tests are to be done, only a few tubes of plasma should be removed from the refrigerator at one time, so that they will not stand at room temperature any length of time before the test is performed. These plasmas should not be placed in the water bath. When performing the test, the plasma should always be added to the test tube first and not last as directed in one of the methods. If added last there is too much danger of contaminating the plasma when repeat tests are made unless a clean pipette is used each time. It is necessary to do at least 2 or 3 tests on each plasma. They must be repeated until two tests check within 0.5 of a second when the prothrombin time is between 12 and 20 seconds, within one second when the time is between 20 and 30 seconds, and within 2 seconds when the time is over 30 seconds. If several technicians in the laboratory are performing the test, they

must be able to check each other within these limits, and this must be done periodically. In order to obtain good checks it is necessary to click the stop watch at the instant the calcium chloride solution is blown into the tube. This is the reason we like a short micro-sugar pipette because it can be held in the mouth, leaving one hand free to hold the stop watch and one to hold the test tube. Also, it is just as important to stop the watch at the fraction of a second that the first fibrin web appears.

Prothrombin determinations should not be made on whole blood. It is possible to check the prothrombin time of whole blood and plasma when the blood is from a normal person, but it is not possible to check whole blood and plasma of the same person when anticoagulant therapy is in progress.

Thromboplastin made of rabbit brain is considered the most satisfactory. That from human brain is too active and should not be used for prothrombin determinations. Difco thromboplastin and Permaplastin are made of rabbit brain. Simplastin is made of a mixture of rabbit brain and lung tissue; while Solu-plastin is made of a mixture of horse brain and lung tissue. Thromboplastin made of lung tissue gives a longer normal prothrombin time because lung tissues contains more blood than brain tissue. The curve made from dilutions of normal plasma is different from that of thromboplastin made of rabbit brain. It is very important to use the dilution curve that comes with the thromboplastin which is used and this should be checked by making at least one dilution of plasma, either the 12.5 or 10 per cent dilution. Obtaining comparable normal control prothrombin times on different batches of thromboplastin does not necessarily mean that the thromboplastin activity curves are superimposable. It is not until the plasma is diluted to 40 per cent or more that the prothrombin time is prolonged to any extent. In dilutions greater than 40 per cent small changes in concentration of prothrombin greatly prolongs the prothrombin time. With the curve for Simplastin this is not as marked because the curve is not as steep at the critical level as that for Difco rabbit brain thromboplastin. This is due to the mixture of lung and brain tissue in Simplastin. It is important that you are consistent in using the same kind of thromboplastin from day to day, and not change from that of one manufacturer to another. It is also important that the curve used corresponds to the seconds received for the normal control. That is, if the normal control is 12 seconds, one curve must be used, if it is 13 seconds a different curve must be used. A formula can be employed when Quick's method is used with rabbit brain thromboplastin and the normal control is between 12 and 15 seconds. This formula cannot be used for other methods or if thromboplastin is made of other tissue than rabbit brain.

$$\text{Per cent prothrombin concentration} = \frac{302}{(\text{P.T. minus X}) \text{ minus } 9}$$

P.T. = Patient's prothrombin time in seconds.

X = Normal control prothrombin time in seconds minus 12.

The thromboplastin must be made and incubated according to the directions for that particular make of thromboplastin. Heating the thromboplastin in a water bath that is one degree higher than the designated temperature will make it inactive. Quick taught us to use a thermos bottle for incubating our thromboplastin and then there is no danger of the water being at too high a temperature, see Fig. 1. A hole is bored into a cork which fits the

Fig. 1. Thermos Bottle Used as Water Bath.

thermos bottle. The tube of thromboplastin is inserted into this hole and then placed in the thermos. A small cork is placed in the top of the hole and the thermos is capped during the incubation. After the thromboplastin is incubated, it deteriorates rapidly at room temperature. If it is not used in one day, it should be divided into tubes according to the amount to be used each day and kept either in the refrigerator or deep freeze. If stored in the refrigerator it will keep two days, if frozen it will keep several weeks. Thromboplastin solution is not only unstable at room temperature but it may vary in its activity because it is made from a crude tissue extract. For these reasons it is very important that a normal plasma be used each day to establish the potency of the thromboplastin. When Solu-plastin is used with dicumarolized plasma, the results do not correspond with those of other thromboplastins; this has also been the experience of other investigators.[2]

The calcium chloride solution should be made up in small enough quantities so that it is prepared once a month. It should also be kept in the refrigerator when not in use. Deterioration is less rapid when kept cold, but sudden deterioration overnight may occur. In the method of Quick, a few ml. of calcium chloride solution are poured into a test tube and placed in the water bath while performing the tests. This solution should be changed after each six or eight tests because it becomes contaminated from the pipette. When the calcium solution is blown into the plasma-thromboplastin mixture, some of this comes in contact with the pipette. Although the pipette is wiped off before placing it back into the calcium solution, we have found out by bitter experience that the solution becomes contaminated after six or eight tests and the prothrombin times are shorter than they should be.

Glassware used for prothrombin determinations should be kept separate and not used for any other test. When it becomes scratched or etched, it should be discarded or used for other purposes. The cleaning of the glassware is one of the most important parts of the test. Each tube should be cleaned with a test tube brush before placing it in cleaning solution because particles of thromboplastin and fibrin web are difficult to remove from the tubes. After being cleaned in acid-dichromate solution, the glassware must be thoroughly washed in tap water and distilled water and then dried. Any acid or alkali remaining in the tubes will interfere with the test. Detergents should not be used because they are more difficult to rinse from the tubes than the acid-dichromate cleaning solution. The size of the tube used in the test is not too important as long as one is proficient in reading the beginning of the formation of the clot, however, for us this seems easier to do with a tube 100 mm. long.

As already mentioned the prothrombin time of blood from a normal person must be determined each day. This serves as a normal control on the solutions for that day's tests. If this value is not within the normal range for the type of thromboplastin used, then one must determine the reason and correct it. Obtaining a normal control is one of the complications of performing prothrombin times. Technicians become tired of being bled and the practice of obtaining blood from supposedly normal patients in the hospital is not a good practice. When a good commercial product is found, it will make this test much more pleasant to do. The commercial products "Protrol" made by the Knickerbocker Laboratories and "Diagnostic Plasma" made by Warner-Chilcott Laboratories, have not proved satisfactory in our hands as they do not check with known normal plasmas.

Prothrombin times should be reported in seconds. If the physicians insist on reports of prothrombin concentration in per cent, then both should be reported. The normal control should be

reported as well as the patient's results. Reports in per cent must be obtained from a curve made for the thromboplastin used. The patient's prothrombin time in seconds can never be divided into the seconds obtained for the normal control because prothrombin concentration does not follow a straight line but makes a hyperbolic curve. If the results are reported in seconds, the therapeutic level is considered to be 2½ times the value for the normal control. When the results are reported in per cent of prothrombin concentration, the therapeutic level is considered to be between 20 and 30 per cent. There is danger of bleeding when the value is lower than 20 per cent.

In summary the following precautions must be taken when performing prothrombin times:

1. Chemically clean and non-etched glassware must be used. The glassware must be kept separate and used only for prothrombin determinations.
2. Solutions must be made fresh each month, deterioration is less apt to occur if the solutions are kept in the refrigerator.
3. A clean puncture of the vein must be made to avoid contamination with tissue juice.
4. The proper proportion of anticoagulant to blood must be maintained.
5. Within 30 minutes after the blood is withdrawn from the patient, it must be centrifuged at medium speed for a short time, the plasma removed and placed in the refrigerator.
6. The test must be done within three hours if oxalate is used as an anticoagulant or within two hours if citrate is used.
7. A normal control must be run each day and if not within the normal range, an explanation must be found and corrected.
8. At least two or three checks must be made on each plasma and they must check within 0.5 of a second for values between 12 and 20 seconds, within one second for values between 20 and 30 seconds, and two seconds for values over 30 seconds.
9. Timing of the clot formation must be exact.
10. Reports should be made in seconds, if made also in per cent of prothrombin activity, the curve for the type of thromboplastin used must be employed.

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PAPER ELECTROPHORETIC SEPARATION

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Recently many articles have been written on paper electrophoresis. Being of the many who have experimented with this sort of thing, I feel it necessary to give suggestions on apparatus, diffusion cells, and technique involved. The apparatus necessarily can be very crude but must be of such a nature that there will be no variables once it is set up. By this I mean voltage and current stabilization, pH controlled in the electrolyte and proper paper.

The power supply must be well regulated as far as voltage and current are concerned. Without this, separation of various ionic fractions *is not* possible. A unit delivering 150-300 D.C. volts potential and 0-40 ma. current with good regulation will be suitabe. This unit can be purchased from Electro Tech Company.*

Current versus time plays an important role in electrophoresis studies. I use about 300 volts at 0.4-0.6 ma. for four hours. Increasing the current increases separation but only separates the strongly charged fractions, eliminating most of the interesting fractions. Alpha and beta globulins are combined with gamma globulin and are never separated out if too much current is used.

The electrolyte cell is an important factor in these studies because the construction must aid in diffusion of the electrolyte. A number of cells have been made and the proper cell is one which lets the paper have the least contact with any surface other than the supporting piece. The diffusion cell electrodes are very important in maintaining electrolyte pH. A soft carbon electrode will cause the cathode electrolyte side to become dark and a drop in pH to the acid side, slowing down separation. Hard carbon rod, or copper and carbon, or platinum electrodes are of choice, the latter being preferable.

Paper for electrophoresis is another determining factor because the coarser the paper, the less accurate the separation because of absorption separation. No. 2 Whatman is preferable or paper of this quality. This can be purchased from any Laboratory Supply house. The width of the paper is determined by the size drop of serum you will be using in the test.

The chief advantage of paper electrophoresis over Tiselius electrophoresis is the simplicity in the construction of the cells and the fact that the cost is very reasonable for results obtained which compare very closely to Tiselius.

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cancer
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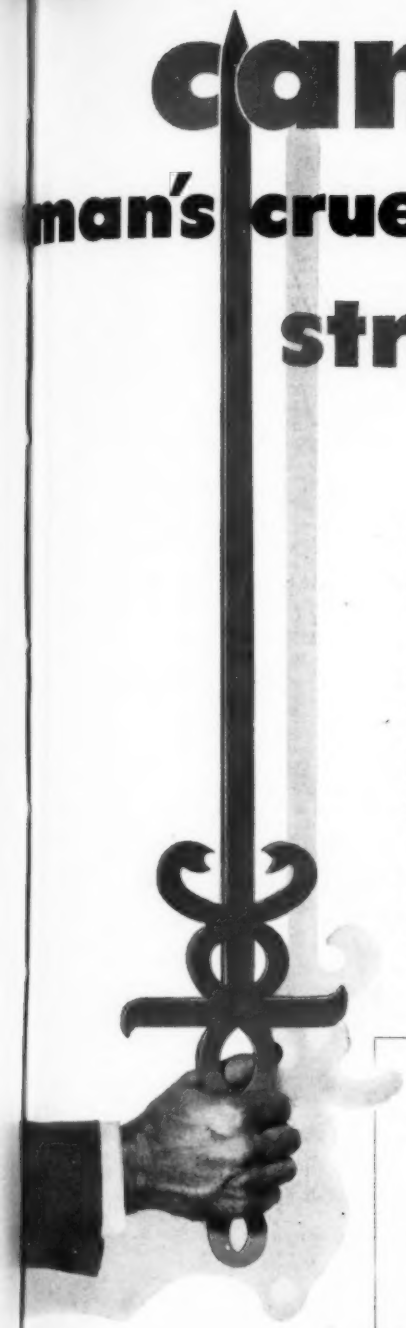

THE FIGHT against cancer is being waged ceaselessly in the research laboratory, in the hospital, the doctor's office. With new methods of diagnosis and treatment, medical science now has weapons to combat man's cruelest enemy more effectively than ever.

THESE LIFESAVING ADVANCES have been made possible by the generous contributions of your fellow Americans. To them the Sword of Hope, symbol of the American Cancer Society's attack through research, education and service to patients, gives assurance of continuing progress today . . . of greater gains tomorrow.

JOIN WITH THEM in striking back with a gift to the American Cancer Society.

American Cancer Society

GENTLEMEN:

☐ Please send me free information on cancer.

☐ Enclosed is my contribution of $..... to the cancer crusade.

Name.....

Address.....

City..... State.....

Simply address the envelope:
CANCER c/o Postmaster, Name of Your Town

AMONG THE NEW BOOKS

THE BOOK OF HEALTH. By R. Lee Clark, B.S., M.D., M.Sc. (Surgery), M. D. Anderson Hospital for Cancer Research, Professor of Surgery, The University of Texas, Post-Graduate School of Medicine, Fellow of the American College of Surgeons, National Consultant in Surgery, U. S. Air Force, and Russell W. Cumley, B.A., M.A., Ph.D., Director of Publications, M. D. Anderson Hospital, Professor of Medical Journalism, The University of Texas, Post-Graduate School of Medicine. Elsevier Press, Inc., Houston, New York. 1953. 836 pages. 1400 illustrations. $10.00.

In an easily understood and entirely "readable" style the BOOK OF HEALTH has a place in every man's library. There is a wealth of illustrations, many of historical, others of current, value. The historical sketches and biographical briefs add considerably to the "readability" of a volume by no means as formidable as some of the earlier attempts at the same result. Rather the "242 doctors" believe you should know something about medicine, and in no uncertain terms bring the layman information he can understand. They take away much of the mystery, and consequently the fear, of medicine.

The book begins with conception and carries through childhood the functions of various parts of the body, in general, goes through the circulatory system, the respiratory system, the skin, the muscles, and the endocrines, and includes the disorders of the mind, as well as a section on home nursing.

The medical technologist will be especially pleased to find his profession referred to in its proper terminology and described in due proportion to those other professions related to medicine.

THE INTELLIGENT USE OF THE MICROSCOPE. 1st American Ed. By C. W. Olliver. Chemical Publishing Company. 1953. 192 pages. 59 illustrations and diagrams. $4.00.

To know the general theories involved in the use of the microscope is tantamount to getting the best use of the instrument. In the chapter on optical theory the basic principles of magnification and various aberrations are given. There are two chapters on the microscope, its components and accessories, and on selecting the right equipment. It is good to find emphasis on the importance of illumination. The comment that it "is essential to know how to obtain the maximum out of the instrument and every one of its components before one can decide where and how to simplify" can be applied not only to this, but to all technics. We liked especially the chapter on the "Microscope in Use." There is a long chapter devoted to photomicrography as well as one on phase contrast, and another on special instruments and their applications.

URINE AND URINALYSIS. 3rd Ed. By Louis Gershenfeld, B.Sc., P.D., Ph.M., D.Sc., Professor of Bacteriology and Hygiene, Director of Bacteriological and Clinical Chemistry Laboratories, Philadelphia College of Pharmacy and Science. Romaine Pierson Publishers, Inc., New York. 1948. 347 pages. 42 illustrations. Appendix.

In one compact volume we have a very complete presentation of the techniques involved in one of the most (so-called) "routine" aspects of the clinical laboratory. There are three main headings beside an appendix giving a brief description of apparatus and reagents. Part I. *General Considerations* has chapters on the functioning of the urinary organs, collection of specimens, physical characteristics, chemical composition and abnormal constituents. Part II includes the qualitative and quantitative tests, as well as a chapter on the microscopic examination of urine. Part III is made up of three chapters on special tests.

Although there is little new presented, this book can be of value in any clinical laboratory.

ELEVEN BLUE MEN AND OTHER NARRATIVES OF MEDICAL DETECTION. By Berton Roueché. Little, Brown & Company, Boston. 1954. 12 short stories from THE NEW YORKER. $3.50.

When you first open this book, whether at THE ELEVEN BLUE MEN, A MAN FROM MEXICO, or any one of the other ten stories, prepare for a full evening of relaxation. Technically correct, these stories are written in a thoroughly entertaining style of a really GOOD detective story. Psittacosis, gout, trichinosis, leprosy, tetanus, and even smog, are the "dirty, dirty villains." I'll say no more, but shall strongly urge you to read ELEVEN BLUE MEN, and all the rest just any evening at the end of a hard day at the laboratory.

ABRAHAM TREMBLEY, Scientist and Philosopher. By John R. Baker, M.A., D. Phil., D.Sc., Lecturer in Zoology and Reader in Cytology in the University of Oxford; joint Editor of the Quarterly Journal of Microscopical Science. Edward Arnold & Co., London. St. Martin's Press, New York. 1952-54. 259 pages. 52 illustrations, many of them the famous Lyonet engravings. $7.50.

Abraham Trembley of Geneva is a great but little known figure in the annals of natural science. His early observations concerning cell division, with discovering that certain animals can be artificially multiplied by division, and his further observations of budding and asexual reproduction, as well as his pioneering steps in grafting animal tissue, make him among the all-time "greats" in the world of scientific discovery. He was a person of diverse interests, with an absorption in politics, religion, moral philosophy, and in methods of education. Many of his principles are applicable today, especially in the last-named field.

It is most fascinating to read of Trembley's discoveries of cell division in the single-celled plants and to compare these latter with the miniscule animal-forms of life he had observed. This biography is close enough to us to prove entirely pleasant reading.

ATLAS OF EXFOLIATIVE CYTOLOGY. By George N. Papanicolaou, M.D., Ph.D., Clinical Professor of Anatomy Emeritus, Cornell University Medical College. Harvard University Press, Cambridge, Mass., Commonwealth Fund. 1954. Loose leaf. 57 pages of general text, 4 figures in text, 36 plates. Bibliography and Index. $18.00.

Many books are published. Many books are good to have in a library, either general or specialized, but only occasionally does a volume appear that a specialist in a given field cannot be without. Any histocytologist will number this book among his most valued possessions. First, it is made up for convenient use in the laboratory—with a minimum of written text and explanation that is concise and without the usual extraneous matter that has to be discarded before the "meat" of the subject is reached. The looseleaf form will lend itself immediately to economical revision in a growing and ever-changing field.

The plates can be described in no less glowing terms. It is a beautiful book with many outstanding plates, each with individual descriptions as well as a few pages of discussion of the cells pictured. When every field of medical technology has a comparable volume, it will be possible to have a complete library with a minimum of books.

Also received:

DISEASES OF THE RETINA. 2nd Ed. By Herman Elwyn, M.D., Senior Assistant Surgeon, New York Eye and Ear Infirmary. The Blakiston Company, Inc., New York. 713 pages. 243 illustrations (20 color illustrations). $12.00.

1954 MEDICAL PROGRESS. A Review of Medical Advances During 1953. Morris Fishbein, M.D., Editor. The Blakiston Co., New York. 19 articles beside summarizing notes. 345 pages. $5.00.

EDITORIAL

ENLARGED PROGRAM FOR RECRUITMENT OF MEDICAL TECHNOLOGISTS

The medical technologist has long recognized the need for more certified members of his profession. Members of ASMT have been engaged in an organized recruitment program for the past eight or ten years and, with the greatly increased work load in the laboratory resulting from the rapid advances in medicine during the past quarter century, have found it necessary to concentrate more and more effort toward seeing that qualified personnel becomes available.

Knowing full well that many of our past methods of attracting sufficient numbers of qualified trainees to fill this demand have been inadequate, largely through a lack of funds and the apparent indifference of others who should have been cognizant of the problem, we were pleased to note that in 1952 the American Society of Clinical Pathologists, whose Registry of Medical Technologists had already extended full cooperation, appointed a Motion Picture Committee to secure funds for the production of a motion picture and telecasts to be used for recruitment at a national level. In 1953 the American Cancer Society allowed $30,000 for the production of a film, and soon afterward the National Cancer Institute made a grant of $15,000 to help achieve full utilization of this material.

At our annual convention in Louisville, Kentucky, last June (1953) the former was announced. Our representative on the Committee had been appointed to serve with representatives from the ASCP and the College of American Pathologists. She will assist in coordinating our existing facilities and plans with the efforts of the other organizations involved in order to achieve the best effect.

The Committee will exist as a national service agency for state and local recruitment programs. It will augment and implement such programs as are already in effect as well as those being organized in our affiliates and their constituent district and local societies. The Committee will find sponsors for producing effective recruitment materials. It will act as a central clearing house for recruitment techniques, bringing to the local groups methods and materials that have proven effective elsewhere through such media as we already have available in our own program and that which is being initiated by the pathologists' organizations. It will attempt to reach nationwide audiences with the program by utilizing national channels of communication. It is hoped that this will eventually result in complete cooperation and coordination between pathologists and medical technologists in their recruitment efforts at the community level. If the gap between supply and demand is to be narrowed, the heaviest share of the work must be done on the local level. Nationwide appeals can only set the stage—the prospective trainees are to be found in the schools and colleges of YOUR city and town, the audiences all recruitment efforts must reach.

A good color film will be made—production is already under way. If it is to be effective, it must be shown to likely audiences. If this has not already been accomplished liaison must be established with the vocational guidance agencies and science teachers in your community, and showings must be scheduled for suitable times. Nor can the film be expected to stand alone. There must be someone present who can answer specific questions. Your state recruitment chairmen have been supplied with kits containing some material of this type that can be adapted to local conditions. Beside the movie, material will be provided for local television stations. Arrangements will have to be made locally for the station manager to put the show on the air and to supply the "live" introduction and conclusion that adapt it to the locale. The work of the

Committee on Careers in Medical Technology is just getting started. Since many of you have already engaged in such programs, your cooperation will be of much help. Please share any information on programs, methods, and materials that have proven effective in your community. Even though some of this is "old hat" to you, the Committee may be able to give it a new "twist." All these efforts will be coordinated with the local, district, state and national societies of medical technologists, and the pathologists' organizations. It's to our mutual advantage to work together. Send your ideas or information to Dallas Johnson, Executive Secretary, National Committee on Careers in Medical Technology, 1785 Massachusetts Avenue, N.W., Washington, D.C., as well as to the ASMT Recruitment Chairman, Sister Barbara Clare Hageman, St. Patrick's Hospital, Missoula, Montana. They will be shared with others through the facilities of ASMT.

WHAT'S IN A NAME?

We claim that MEDICAL TECHNOLOGY IS A PROFESSION—is it? What picture appears when we hear a doctor referred to as "DOC"? —What then, do we expect to accomplish by calling ourselves "MED TECHS" or "techNICIANS"?

Pause a moment before calling yourself by a term less than the professional title you have earned. Saying **"MEDICAL TECHNOLOGIST"** can become a habit—and know that by using the full phrase you will have added just a little more dignity to our profession. Remember, one must have self respect before others will respect him.

IN MEMORIAM

The Maryland Society of Medical Technologists announces with deep sorrow, the death of Sr. M. Joan of Arc, R.S.M. of Mercy Hospital, Baltimore, Maryland, on January 5, 1954.

Sr. M. Joan was the organizer and first president of the Maryland State Society of Medical Technologists. She was also one of the first members of the National Society.

ANNOUNCEMENTS

NOMINATIONS AND ELECTIONS COMMITTEE REPORT

The following persons will be presented for the vote of the House of Delegates in annual session June 15-16, 1954, at Miami Beach, Florida.

PRESIDENT-ELECT: (one to be elected)

BARBARA ISBELL, California: Member AMST since 1946. ASMT Board of Directors 1951-54. Advisory Council 1949-54. Standards and Studies Committee 1947-49. Legislation Committee 1949-52. Board of Directors Handbook Committee Chairman 1952-53. Board of Directors Executive Office Site Investigation Committee Chairman 1952-53. Board of Directors Executive Office Committee Chairman 1953-54. Delegate to ASMT conventions 1949-54. President California Society of Medical Technologists 1949-50. Secretary 1951-53. Chairman of various committees in state society.

MRS. ELINOR JUDD, Massachusetts: Member ASMT since 1946. ASMT Board of Directors 1951-54. Advisory Council 1949-54. Delegate ASMT conventions 1949-54. Standards & Studies Committee 1949-52. General Chairman Convention 1951. President Massachusetts Association of Medical Technologists 1949-51. Member of various committees in state society.

RECORDING SECRETARY: (one to be elected)

SISTER EMERITA OHMANN, Minnesota: Member of ASMT since 1946. Secretary Minnesota Society of Medical Technologists 1949-50, 1953-54.

SISTER MARY SIMEONETTE SAVAGE, Kentucky: Member ASMT since 1949. Recording Secretary 1953-54. Member Editorial Staff of ASMT 1952-54. Chairman Program Committee Convention 1953. Won 1st award for paper presented 1949 convention. Delegate ASMT conventions 1949-54. Member Advisory Council 1951-52, 1953-54. President Kentucky State Society of Medical Technologists 1951-52. Chairman of various committees in state society.

TREASURER: (one to be elected)

KATHRYN DEAN, Maryland: Member of ASMT since 1945. Chairman Finance Committee 1952-54. Advisory Council member 1950-51. Delegate ASMT conventions 1950-51. President Maryland Society of Medical Technologists 1950-51. Board of Directors MSMT 1947-48.

MOLLIE HILL, Virginia: Member ASMT since 1938. Delegate to ASMT conventions 1939-42, 1944, 1947-48, 1951. President Western Pennsylvania SMT 1939. President D. C. Society of Medical Technologists 1946-47.

BOARD OF DIRECTORS: (two to be elected)

MARY FRANCES GRIDLEY, Virginia: Member ASMT since 1947. Associate Editor AJMT 1953-54. Chairman Research Committee 1953-54. Advisory Council 1950-51. Delegate to ASMT conventions 1948-53. President D. C. Society of Medical Technologists 1950-51.

ANNA BELL HAM, Florida: Member ASMT since 1949. Advisory Council 1951-53. Delegate ASMT conventions 1950-54. Chairman Program Committee convention 1954. Standards & Studies Committee member 1952-54. ASMT representative on ASCP Reclassification Study Committee 1953-54. President Florida Division of ASMT 1951-53. Chairman various committees in state society. Board of Directors state society 1949-51 and 1953-54.

MRS. ELSA KUMKE, Michigan: Member ASMT since 1948. Delegate to conventions 1952-53. Member Advisory Council 1953-54. ASMT representative on ASCP Reclassification Study Committee 1953-54. Legislation Committee 1952-54. President-elect Michigan Society of Medical Technologists 1953-54. Chairman Investigation Committee of Int. Standard Training 1951.

AUDREY MURPHY, Missouri: Member ASMT since 1947. Member Advisory Council 1952-54, Recording Secretary 1953-54. Secretary Missouri Society of Medical Technologists 1951-52. President-elect 1952-53. President 1953-54. Editor *MSMT Newsletter* 1950-51. Chairman various committees state society.

BOARD OF APPROVED SCHOOLS: (one to be elected)

SISTER ANNA CECELIA BRENNAN, Missouri: Member ASMT since 1948. Board of Directors Mo. S.M.T., Chairman various committees state society, including Education Committee. 20 years teaching experience, in approved school for Medical Technologists.

MRS. HAZEL CURRENT ROGERS, California: Member of ASMT since 1944. Delegate to ASMT conventions in 1948, 1950, 1951. Board of Directors California Society of Medical Technologists 1951-52. Member various committees state society. Was High School teacher for 13 years and principal of High School for 9 years. Holds rank of Training Officer in V.A. Hospital where she is in charge of an Approved School for Medical Technologists.

SCIENTIFIC PRODUCTS FOUNDATION AWARD

The Scientific Products Foundation offers awards of $100, $50 and $25 in each of seven classifications for papers selected by an ASMT Awards Committee. The awards will be announced at the annual ASMT convention and will be granted to the writers of the three most outstanding papers in each of the following categories.

Blood Banking	Hematology	Bacteriology
Blood Typing	Histology	
Blood Chemistry	Serology	

Award papers will be chosen on the basis of their contribution to the improvement of clinical laboratory technique or the improvement in accuracy, with particular emphasis on simplification and the encouraging of exchange of time saving suggestions.

Papers eligible for 1954 awards include:

(1) those written by a member of ASMT, and submitted in triplicate, typewritten, double-spaced, on paper 8½ x 11" and not previously published.

(2) those written by an ASMT member, and published in the *American Journal of Medical Technology* since Jan. 1, 1950. (These will automatically be reviewed and considered for the 1954 awards.)

(3) those written by an ASMT member and printed in an affiliated state society publication since Jan. 1, 1950, may be submitted as a reprint or typewritten, in triplicate, double-spaced, on paper 8½ x 11".

(4) those written by an ASMT member and published in any recognized scientific publication will be considered if submitted as a reprint.

(5) those papers written by students as part of their training in an approved school of medical technology. Any unpublished material, including papers written by students must be accompanied by certification of authorship by the institution with which the author was connected during the writing.

In case of co-authorship, the award will be presented to the ASMT member(s). All papers will be coded **prior to judging.**

Papers written by students must be submitted by May 1, 1954, to receive consideration this year.

All papers will be transmitted to the Awards Committee through
Miss Rose Matthaei, M.T. (ASCP), Executive Secretary
Suite 25, Hermann Professional Building
Houston 25, Texas

All papers must be typewritten on paper 8½" x 11", with two carbon copies. The decision of the Awards Committee will be final and all papers and copies become the property of ASMT.

To assure complete impartiality and objectivity in considering papers for awards, the Scientific Products Foundation Awards Committee will consist of a medical technologist (ASCP), a clinical pathologist well versed in the subject to be judged and an additional member who may or may not be a member of either ASCP or ASMT, but must be a specialist in the selected field.

The Scientific Products Foundation is a non-profit corporation supported by the Scientific Products Division of American Hospital Supply Corporation and associated companies—Coleman Instrument Company, Dade Reagents, Inc., Hartman-Leddon Company and C. A. Hausser and Company. It is the purpose of the Foundation to encourage better laboratory work by facilitating exchange of new or improved methods and techniques between laboratories and by providing special recognition for individuals who make meritorious contributions to the laboratory field and its practicing professionals.

ANNOUNCEMENT

The 31st Annual Conference of the American Physical Therapy Association will be held at the Hotel Statler in Los Angeles, California, June 27-July 2, 1954.

CATHOLIC HOSPITAL ASSOCIATION CONFERENCE

The Catholic Hospital Association is again sponsoring a three-day Conference for medical technologists in conjunction with its annual convention, which this year will take place in Atlantic City, May 17-20. The dates for the Medical Technology Conference are May 17, 18 and 19.

The program will consist of four 2-hour sessions, beginning on Monday afternoon, May 17, and ending Wednesday morning, May 19. The first session will be devoted largely to education, both from the standpoint of educational advancement for medical technologists and from that of medical technology schools. The latter topic will be treated in the form of curriculum discussion.

The Tuesday morning, May 18, session will be of a technical nature, with a discussion of new developments in blood banking and a presentation of the theory and technique of 17-Keto steroids. The afternoon session will be particularly devoted to hospital laboratory policies, from an administrative viewpoint. A business session with elections will conclude the day's program.

The concluding meeting, on Wednesday morning, May 19, is again of a technical nature, with emphasis on calibration. In all meetings provision has been made for ample discussion time, and the Committee on Medical Technology of The Catholic Hospital Association will be available to give all possible assistance to registrants. The following Sisters are on this Committee:

Sister Mary Clare, **Chairman** 1953-54, St. Clare's Hospital, New York, N.Y.; Sister Mary Emerita, St. Gabriel's Hospital, Little Falls, Minn.; Sister Mary Leo Rita, St. Mary's Hospital, Madison, Wis.; Sister Charles Adele, St. Vincent's Infirmary, Little Rock, Ark.; and Sister Mary James, Santa Rosa Hospital, San Antonio, Tex.

POSTER CONTEST WINNERS

Winner of the first prize, a clock radio, in ASMT's Poster Contest is Miss Grace Paul, M.T. (ASCP) of Hutchinson, Kansas. The second prize of a portable radio went to Sr. M. Edwin (Kenna) of Mercy Hospital, Pittsburgh, Pennsylvania, and the third prize of a Parker pen to Sr. M. Mauritia (Schuermann) of Sacred Heart Hospital, Yankton, South Dakota. Previously it was announced that honorable mention would go to five participants. The judges are listing eight Honorable mentions but not in order of their preferences so congratulations to the following: Miss Esther Wilbrecht, New Ulm, Minnesota; Miss Barbara Straw, Fitchburg, Massachusetts; Miss Carol Warwick, Galveston, Texas; Miss Dorothy Lozoski, Amherst, Massachusetts; Miss Marjorie Bailey, Youngstown, Ohio; Miss Jewell Mitchell, Atlanta, Georgia; Sr. Mary John (Marzluf), Nelsonville, Ohio, and Sr. Charles Adele (Wells), Little Rock, Arkansas.

The judges were Miss Muriel Wageman, advertising consultant to educational and institutional advertisers; Frank Manning; Commercial and illustrative artist, President of Manning Enterprises, Unlimited, Commercial and illustrative artists and Miss Ruth Feucht, past Chairman of Recruitment Committee for ASMT. A fourth invited judge was a pathologist, Dr. Coye Mason of Grant Hospital, Chicago, who was unable to attend at the last minute. Present at the judging ceremonies were Mr. R. J. Mayer; Lewis Glassner of the Glassner & Associates, Public Relations; E. Skir-

Top: Some of the entries.
Bottom: Two judges: Frank Manning, Miss Wageman, and the Winning Entry.

mont Diffenbaugh, Chairman of ASMT Public Relations Committee officiated at the ceremonies but did not judge.

The quality of the entries was exceptionally high in the opinion of the judges who engaged in a four-hour session before making a final decision. The judging committee expressed its pleasure at finding so many entries to which extreme thought and knowledge of recruitment problems had been applied. Most of the posters submitted were colorful and attracted attention through the messages portrayed.

It is the plan of the Public Relations Committee that Miss Grace Paul's winning entry be used for reproduction for national distribution. It has been slightly altered in order to project a finished art work poster that can be reproduced in quantity and distributed to hospitals, laboratories and schools throughout the country. These posters will be available through ASMT headquarters in time for participation in National Hospital Week in May for Laboratory Open House.

ABOUT YOUR CONVENTION PROGRAM FOR JUNE

Your program committee hopes that the speakers and the subjects for this year's scientific program will be varied enough to please technologists in every field. In the last issue we gave you a partial list of speakers. Now we would like to give you a more detailed outline of what's in store for you. We'd especially like to call attention to the following subjects as being —we think—in a "new and different" line:

Dr. Theodore E. Weichselbaum, of the Washington University School of Medicine, St. Louis, is to give a workshop on Instrumentation, with demonstrations on the instruments under discussion. This will be offered at least twice, and possibly three times, in order to accommodate all those who will want to hear him. We feel that this is a "must" for you who work with colorimeters or photometers of any kind, and is being given by popular request. (Those who wish to attend this workshop please note form to be sent in by **APRIL 15.**)

Dr. E. W. Cullipher, Orthopedist, of Miami, is going to talk on Bone Banks—a new field in which all will be interested.

Dr. C. B. Pollard of the University of Florida has chosen "Developments in the Fields of Venoms, Antivenoms, and Snakebite Treatment." Those of us in Florida who have heard him several times on this subject want to advise you in advance that he is a fascinating speaker; and whether you feel that this is in your "line" or not, you'll come away with an education on the subject.

Dr. Robert Boucek of the Miami Heart Institute will present some original research on which he has been working. The title of his paper is "The Expanding Horizons of the Arteriosclerosis Problem."

From Columbia University Medical School comes Dr. H. W. Brown who will speak on "Amoebiasis," and while the subject may not be new, anything Dr. Brown touches assumes a new aspect—just ask anyone who has had the pleasure of hearing him! Dr. Brown also is to be our banquet speaker. Title? "Medical Serendipity." You don't know what that is? Well, we're mighty curious too, and it seems there is only one way to find out.

Many of our own members will speak too. Space won't permit a complete listing, but we would like to tell you that Miss Genevieve Stout of the Venereal Disease Laboratory in Chamblee, Georgia, will present a paper entitled "Developments and Trends in the Serodiagnosis of Syphilis."

If we haven't mentioned your particular field yet, it will be there. We will have hematology from Dr. Sherman Kaplan of Miami Beach; bacteriology from Dr. Vincent Saurino of the University of Miami School of Medicine; and biochemistry from Dr. George T. Lewis, also of the University of Miami School of Medicine. Nor is that all. We do hope, however, it is sufficient inducement to bring you here so that you may also hear the rest of our speakers.

And—oh, yes—workshops! Plans for these have not yet been completed, but we're trying to include all the ones you requested. Some of these will be in the form of luncheon meetings; others will be night workshops. One thing we can promise you: There will be one on State Publications. This is the workshop most requested by members—so come and get the most out of it.

SEE IT AT MIAMI BEACH

CONVENTION ANNOUNCEMENTS

MIAMI BEACH AND GREATER MIAMI IN JUNE

The 22nd Annual Convention of ASMT at Miami Beach, Florida, will begin June 12 at 2:00 P.M. with registration in the lobby of the Delano Hotel. Registration will continue until 5:00 P.M. and will be resumed Sunday at 8:00 A.M. The Advisory Council meeting will begin at 9:30 A.M., by request. A church directory will be available at the registration desk.

At 1:30 P.M. (Sunday) you will have a choice of one of three tours—two by land and one by sea. There is a grand or combined tour which includes the beaches, Hialeah Race Course, a Seminole Indian village, the University of Miami campus, Coral Gables, the City Beautiful, and the Orange Bowl among other places. All of this takes approximately four hours. The second trip by land is somewhat shorter, taking about 2¾ hours, and covers the beaches, Hialeah and the International Airport. The boat cruise will take you through the inland waterways and give you a glimpse of the lovely homes on the man-made islands surrounding Miami Beach, which is itself an island. Hialeah, besides being what many consider the most beautiful race track in the world, is the home of a flock of flamingoes, that striking pink-feathered bird of the tropics. They live on islands in the lake made for them in the centerfield of the track. The University of Miami's new campus is a triumph of modern architecture and has been built, entirely, since World War II. International Airport is one of the largest and busiest in the United States.

On Sunday night at 8:00 the Florida Division of ASMT invites all to attend the reception to be held on the Terrace at the Delano Hotel.

Monday, June 14, at noon we hope you will enjoy the Cabaña Luncheon and Watershow at the Shelborne Hotel. Here you may see pool and ocean at the same time. And do bring your sunglasses—that sun can be mighty bright during the day!

For those of you not attending the House of Delegates sessions, Tuesday afternoon and Wednesday morning will be free. Arrangements may be made for any of the aforementioned tours or for any of the many other interesting trips available—Villa Vizcaya, Parrot Jungle, the Rare Bird Farm, Monkey Jungle, etc. Or maybe you'd like to go deep-sea fishing. That, too, can be arranged. And there is always shopping on famed Lincoln Road. But be sure you finish these extra-curricula activities before Wednesday afternoon because we think you won't want to miss the Hawaiian Luau (feast) at Crandon Park. Wear your bathing suits under your shorts or slacks if you want a swim, but take along a jacket for it can get cool after the sun goes down. There will be a full moon—and a surprise.

The banquet, which is to be INFORMAL, will be at 8:00 P.M. Thursday in the ballroom of the beautiful (and brand new—it opened in December) di Lido Hotel. As you will note elsewhere, Dr. H. W. Brown will be our speaker. This ends what we are confident will, with your participation, be one of the most successful conventions ASMT has ever held.

Mrs. Maxine Ace
Mrs. Anna Louise Rundell
Co-chairmen of Publicity

GENERAL INFORMATION

Florida in June is sun-warmed to about 80° with a pleasant ocean breeze to stir the palm fronds. In order to enjoy this to the fullest, bring informal clothes, cottons and comfortable shoes for the women and slacks and sport shirts for the men, sun-glasses for all. Since the hotels all have pools as well as the ocean, you'll want to bring bathing suits. And don't forget the shorts, slacks or playsuits for the Crandon Park trip. But PLEASE, do be cautious in acquiring that sun-tan! It is not at all unusual for visitors to be hospitalized with sunburn. And before we forget—since the hotels and

Auditorium are air-conditioned and the evenings sometimes cool, you may wish to bring a sweater or a light jacket.

Since Miami Beach is an island it has no stations, whether you arrive by plane or train, your destination will be Miami. There is limousine service from the Airport to the Beach. This is more reasonable than taxi. From either of the two train stations, taxis are available.

Or are you planning to come by car? Route 41 comes down the West Coast through Land O'Lakes, Sulphur Springs, Tarpon Springs (sponge fisheries), St. Petersburg, Tampa, Sarasota (winter quarters of Ringling Brothers Circus, also home of the Ringling Art Museum) and Fort Myers (where you can see Edison's workshop); then across the Tamiami Trail through the heart of the Everglades and so to Miami. The most scenic route, perhaps, is 441-27 and 301 through the central part of the state, where you will find Ocala and Silver Springs, Winter Haven and the Bok Tower, the orange groves and Cypress Gardens. Or perhaps you prefer Route 1 through Jacksonville; St. Augustine, the oldest city in the United States; Marineland between St. Augustine and Daytona Beach; and the famous beach at Daytona Beach itself. If you have your car you will want to go on South from Miami to Key West over the famous Overseas Highway. It is here you will reach the Southern-most point in the United States, only a short distance from Cuba.

Would you like to go to Cuba, Nassau, Jamacia, Haiti, or Puerto Rico? Just read all the forms and check in the appropriate places and information will be sent to you. We want you to enjoy your Convention and then go on to enjoy a tropical vacation. But beware—you'll get sand in your shoes and want to come back.

SCIENTIFIC - TECHNICAL EXHIBITS

Please reserve booth space for your scientific or technical exhibit as early as possible. Prepare your exhibit for the 1954 ASMT Convention and write to Miss Emelia M. Lanzetta, 3214 Liddy Avenue, West Palm Beach, Florida, for a reservation.

The backwall of the booth will be blue-gray flameproofed draped material with side dividers covered with the same material as the backwall. A table is furnished. Covering for the table is to be furnished by the exhibitor.

Instructions for shipping will be sent after the requests are received.

Please check the following and return to Miss Lanzetta at the above address.

1. Size of booth. All booths are 10 ft. x 10 ft.
2. Counter table. Needed: Yes No
3. Chair. Needed: Yes No
4. Electric outlet. Indicate if needed for display.
   One will be furnished if indicated. Additional outlets $7.50.
5. Headboard sign. 9 in. x 44 in. One or two lines to read

PLEASE PRINT

Applicant responsible for exhibit: _____
(Signature)

N.B. Please print:
Name of Applicant
Address of Applicant
Type of Exhibit

SPECIAL FORM FOR INSTRUMENTATION WORKSHOP

In order to know how many times the workshop in Instrumentation must be given, will you please check and return this form if you plan to attend? At the session to be held before the House of Delegates meetings start, delegates will be given preference. Please return this form by APRIL 15 to:

Anna Bell Ham
1190 South Alhambra Circle
Coral Gables, Fla.

We realize that many of you will not know by April 15 whether you will be a delegate or not, but we must know by that date just how many of you plan to attend. You will be given a later opportunity to tell us whether or not you are a delegate. You may put the form on a post-card if you wish.

I plan to attend the workshop on Instrumentation.

I am_____ am not_____ (don't know_____) a delegate.

Name:_____

Address:_____

SPECIAL FOR THE SISTERS

A special trip has been arranged on Sunday to give you a chance to see Mercy Hospital, which is located on part of the old Deering Estate and faces Biscayne Bay, and Villa Viscaya, former home of James Deering. Villa Vizcaya, now an art museum, is modeled from a 16th Century fortress-like structure and is surrounded by Italian gardens. It houses a wonderful collection of paintings and art objects from far-away places. Mr. Deering traveled for 25 years through Europe collecting rare textiles, period furniture, sculpture and ceramics. The Italian art is unexcelled in America.

On Wednesday St. Francis Hospital has planned a buffet dinner followed by a movie. This hospital has a fine view of Indian Creek as well as the ocean, and is situated on Allison Island at 63rd Street, Miami Beach.

HOUSING FORM FOR SISTERS

Date of Arrival:_____

Time: A.M._____ P.M._____ After 6:00 P.M._____

Date of Departure:_____

I would like: Convent Accommodations_____

Hotel Accommodations_____

Name:_____

Address:_____

Please return as soon as possible to:

Sister Evelyn Marie
St. Francis Hospital
Miami Beach, Florida

COME TO MIAMI BEACH IN JUNE
(June 13-17, 1954)

In order to facilitate making plans for what we hope is one of the most successful A.S.M.T. conventions, we are presenting a hotel reservation form. The success of the meeting is dependent upon your participation so plan early to attend. All reservations will be made through the housing committee. PLEASE RETURN THIS FORM BY JUNE 1 AS WE CAN NOT GUARANTEE HOUSING AFTER THAT DATE.

The Hotels Delano and di Lido are joint headquarters. Since everyone cannot be accommodated in these hotels, it would be appreciated if members would arrange to share rooms or indicate a willingness to be assigned a roommate by the housing committee. In case of two persons making reservations together, confirmation will go to the first one making the request. No deposit is required for hotel reservation. Be specific about your arrival date.

Date of Arrival:_____

I will arrive by Air, via_____Airline.

Train, via_____Railroad.

Bus_____, Private car_____.

Time: A.M._____P.M._____After 6:00 P.M._____

Date of Departure:_____

Name:_____

Address:_____

Since delegates will be placed at the Delano and di Lido, will you please check which of the following you are:

1) member _____
2) delegate _____
3) advisory council _____
4) board member _____

Please indicate accommodations desired:

Rooms: Single ($8 or $10)_____ Double ($8 or $10)_____

Suites ($15)_____

If no room is available at the rate requested, please check here_____ if the next higher rate will be agreeable.

Special rates have been secured for Sisters.

Reservations may be made with the Chairman of Housing,

Miss Helen Partin
5801 N. E. 4th Court
Miami, Florida

In order to facilitate local handling of reservations we would appreciate it if our colored members would contact Mrs. Adele Mason, Jackson Memorial Hospital, Miami, Florida.

I would like information on a pre-_____or Post-convention Trip_____ to Cuba_____; Nassau_____; Jamaica_____; Puerto Rico_____; the West Indies_____; car_____ or bus_____trip to Key West or through the state of Florida.

NOTICE

Guests (family or friends) will be welcome at any of our entertainment features. However, at any event or on any tour where space is limited, members and visiting technologists will be given preference, and guests will be accommodated in the order in which their reservations are received. Our Housing Committee will be glad to help in finding accommodations for your guests. Please be sure to indicate clearly on all reservation forms whether they are for members, non-member technologists, family or friends. We sincerely hope we will be able to accommodate all who wish to attend.

ADVANCE REGISTRATION FORM

This plan is promoted to save YOU time in the Registration Line, thus giving you more time to see "America's Tropicland." After filling out your Registration Card and having your credentials checked, you may pick up your Ready Prepared Kit at the table indicated, ADVANCE REGISTRATION. Remember to bring your current ASMT membership card as identification.

READ CAREFULLY: Every technologist, member or non-member, must pay the registration fee. Guests (family or friends) do not pay this fee. Check each activity you wish to attend and please check in the appropriate columns if you are including payment for non-members or for guests. Make checks or money orders payable to the American Society of Medical Technologists and return with your Housing reservation either to Miss Partin or to Mrs. Mason, as indicated.

		Technologist Member	Non-Member	Guest (No.)
SUNDAY, June 13				
1:30 P.M. Sightseeing Tours				
Combined bus tour	$3.00	____	____	____
Short bus tour	1.73	____	____	____
Boat cruise	2.15	____	____	____
Special trip for Sisters, including entrance fees for Villa Vizcaya	3.30	____	____	____
MONDAY, June 14				
Noon Cabana Luncheon	3.50	____	____	____
WEDNESDAY, June 16				
4:30 P.M. Hawaiian luau, including transportation	4.50	____	____	____
4:30 P.M. Special for Sisters: Buffet supper and movie at St. Francis Hospital, transportation only	0.50	____	____	____
THURSDAY, June 17				
8:00 P.M. Banquet, di Lido Hotel	7.50	____	____	____
REGISTRATION FEE	3.00	____	____	____
TOTAL				

My check____ money order____ for $_____ is enclosed.

ADVANCE REGISTRATION WILL CLOSE JUNE 4, 1954. After that date you may register on your arrival at the Convention.